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8521 C*

Bactericidal Action of Sea-Water.

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It has been shown by the author[1] that sea-water inhibits the multiplication of freshwater bacteria while marine bacteria require sea-water media for their initial isolation, although these differences rapidly disappear following the laboratory cultivation of the bacteria. Similar observations on the selective bacteriostatic action of sea-water have been reported by Berkeley,[2] Lipman,[3] and Korinek.[4]

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* P represents a preliminary, C a complete manuscript.

[1] ZoBell, C. E., and Feltham, C. B., *Proc. Fifth Pac. Sci. Congress*, 1933, **3**, 2097.

[2] Berkeley, C., *Trans. Roy. Soc. Canada*, 1919, **13**, 15.

[3] Lipman, C. B., *J. Bact.*, 1926, **12**, 311.

[4] Korinek, J., *Centralbl. f. Bakt.*, Abt. II, 1927, **71**, 73.

It is generally known that intestinal and other bacteria of public health interest do not survive very long when emptied directly into the sea unless there is appreciable organic matter present or considerable freshwater dilution. Colon bacilli are rarely recovered from the open sea and they occur far less frequently in the vicinity of sewage effluents than can be accounted for by dilution or oceanic circulation. The failure of freshwater bacteria to survive in the sea has been attributed by various investigators to predacious protozoa, lack of nutrients, bacteriophage,[5] or to the lethal effect of sunlight. The following experiments reveal that sea-water *per se* is bactericidal.

In the first series of experiments sewage was appropriately diluted with "formula C"† and 1.0 cc. quantities of the resultant dilutions were transferred to 99 cc. water blanks consisting of different kinds of sea-water as well as a "formula C" control. After thorough mixing by shaking, 1.0 cc. quantities of the final dilutions were plated with nutrient agar. About one minute elapsed from the time the final dilutions were made in sea-water until the agar was poured. Additional 1.0 cc. samples were plated after 15 minutes and again after 30 minutes, and at 30-minute intervals thereafter. The plates were incubated 4 days at 37°C. and the colonies counted. Table I summarizes typical findings on the death-rate of sewage bacteria in sea-water dilution waters. The results are expressed as ratios on a basis of the number of bacteria developing from the "formula C" control being 100.

It will be observed from Table I that sea-water rapidly kills sewage bacteria although 5 to 10% sea-water favors their prolonged survival. Similar results have been obtained with the autochthonous microflora of milk, washings from the hands and buccal cavity, tap-

TABLE I.
Relative Numbers of Sewage Bacteria Which Survived in the Various Dilution Waters for the Stated Period of Time.

	Time in Minutes					
Dilution water	1	15	30	60	90	120
"Formula C" control	100	96	99	102	100	106
Autoclaved sea-water	85	76	51	43	42	34
5 % "	100	98	103	106	99	103
10 % "	102	101	109	106	102	104
25 % "	96	86	82	73	70	72
50 % "	88	81	72	68	69	60
75 % "	81	70	58	45	42	39
Berkefeld-filtered sea-water	81	47	29	18	14	11

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[5] Fernand, A., Sempe and Chavanne, *Bull. Acad. de Med.*, 1925, **93**, 184.

† "Formula C" is a balanced salt solution found to be superior to other dilution waters tried by Butterfield, *J. Bact.*, 1932, **23**, 355.

water, and lakes. Likewise stock cultures of various freshwater bacteria are gradually killed when suspended in sea-water but the death-rate of laboratory acclimatized cultures is much slower than that of similar bacteria which occur in nature. Thus results obtained by suspending stock cultures of *Bact. coli* and human pathogens in sea-water have little public health significance.

Moreover, sea-water which has been autoclaved is less bactericidal than sea-water which has been sterilized by Berkefeld filtration. This is attributed to the thermolability of certain germicidal properties of sea-water, because Berkefeld-filtered sea-water which has been heated behaves as ordinary autoclaved sea-water. Kiribayashi and Aida[6] noted that cholera vibrios live longer in boiled than in raw sea-water but they attributed it to harmful saprophytes.

Evidence that unaltered sea-water has a greater germicidal potency than either heated or Berkefeld-filtered sea-water was obtained by submerging the bacteria in the sea confined in Coors porous porcelain filter tubes impregnated with collodion. Different kinds of freshwater bacteria were suspended in autoclaved sea-water and immediately transferred to the semi-permeable tubes. After being stoppered, some of the tubes were lowered into the sea at the end of the Scripps Institution pier, some were submerged in autoclaved sea-water and some in Berkefeld-filtered sea-water. All were held at the same temperature. At 30-minute intervals the tubes were opened aseptically and 1.0 cc. samples were plated. Table II summarizes representative data. The results are expressed as ratios on a basis of 100 representing the number of bacteria which were recovered from the "formula C" control in semi-permeable tubes.

TABLE II.
Relative Numbers of Sewage Bacteria Which Survived in Sea-water in Semi-permeable Tubes Submerged in Natural, Autoclaved and Berkefeld-filtered Sea-Water.

Treatment	Time in Minutes 30	60	90	120
"Formula C" control	100	91	82	97
Natural sea-water	47	31	15	3
Autoclaved "	56	38	27	36
Berkefeld-filtered sea-water	39	20	23	19

Within the limits of the experimental error the results obtained with autoclaved sea-water and Berkefeld-filtered sea-water under these conditions are not unlike the findings in the previous experiment. However, after the first hour the death rate of freshwater bacteria was much greater when submerged in the sea than when submerged in autoclaved or Berkefeld-filtered sea-water. Giaxa[7] observed that pathogens soon perish in raw sea-water although they may survive indefinitely in sterilized sea-water.

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[6] Kiribayashi, S., and Aida, T., *U. S. Pub. Health Eng. Abst.*, 1934, **14**, 61.

This seems to indicate that raw sea-water possesses some constituent which exerts a bactericidal effect besides its salts. Further proof was forthcoming by suspending bacteria in synthetic sea-water and in isotonic NaCl solutions. It was found that the death-rate was somewhat greater in the 3.0% NaCl solutions than in synthetic or in autoclaved sea-water, probably due to a better mineral balance in the latter, but in Berkefeld-filtered sea-water and more particularly in raw sea-water (submergence experiments) the death rate was greater than in the NaCl solution. In fact, sewage bacteria survived longer in 6.0% NaCl (approximately twice the salt content of sea-water) than they did in raw sea-water. It is believed that the bactericidal potency of raw sea-water is even greater than is indicated by these experiments in which the bacteria were surrounded by the semi-permeable walls of the retaining tubes.

Corroborating the conclusions of Burke and Baird,[8] it has been demonstrated that most freshwater bacteria can be acclimatized to tolerate and actually to grow in sea-water media although many of the bacteria undergo other physiological and in some cases morphological transformations. The addition of organic matter reduces the bactericidal potency of sea-water.

8522 P

Influence of Posterior Pituitary Extracts on Sodium Balance in Normal Subject and in Patient with Diabetes Insipidus.

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From the Scripps Metabolic Clinic, La Jolla, California.

In the normal subject anti-diuretic extracts of the posterior pituitary cause an increase in the excretion of sodium and chlorine with the result that the balance for these elements becomes negative.[1, 2] In Fig. 1 we have presented data on a normal subject (male, 25 years old) which shows the same thing. The patient with diabetes insipidus (male, 19 years old), on the other hand, behaves differently. The same dose of posterior pituitary extract as given the normal caused no increase in sodium chloride excretion although a

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[7] Giaxa, D., *Z. f. Hyg.*, 1889, **6**, 162.

[8] Burke, V., and Baird, L. A., *J. Bact.*, 1931, **21**, 287.

[1] Manchester, R. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 717.

[2] Engel, R., McQuarrie I., und Ziegler, M., *Arch. f. Exp. Path. u. Pharm.*, 1933, **173**, 248.

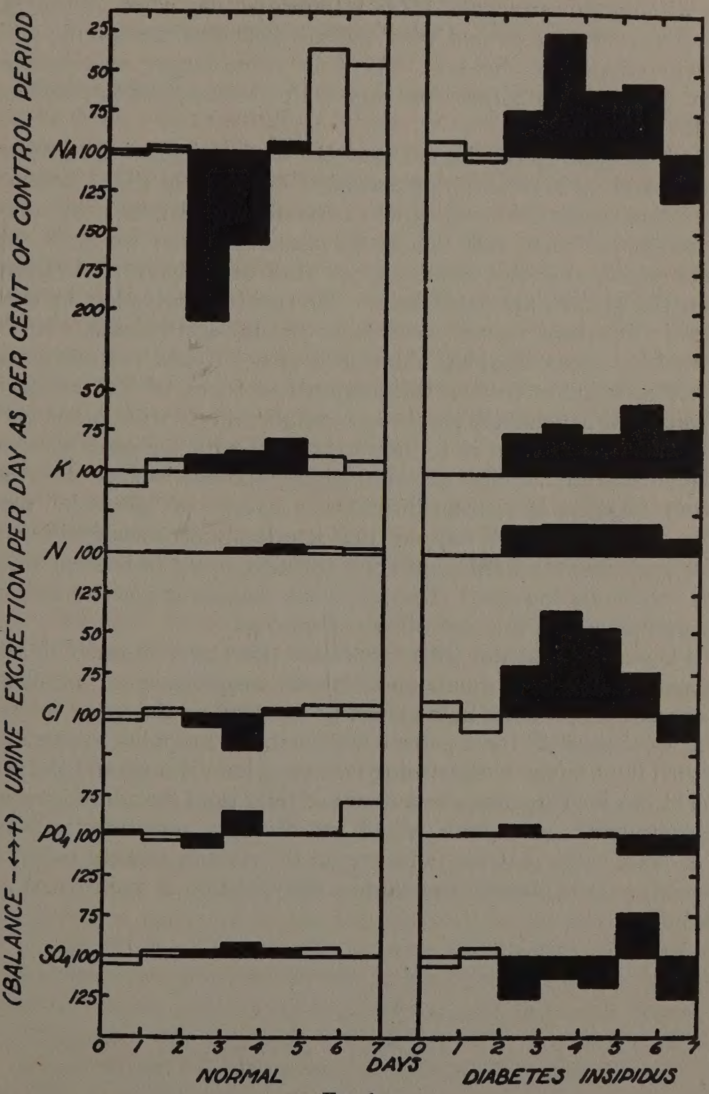

FIG. 1.

marked anti-diuresis resulted (Fig. 1). The days upon which "Pituitrin" (Surgical, 0.5 cc. twice daily) was administered are indicated in solid black. In the normal subject there was no appreciable reduction in urine volume the rate being about 1.5 liters per day without the extract and 1.2 to 1.4 liters per day when "Pituitrin" was given. In the patient with diabetes insipidus the specific anti-diuretic effect was obvious. The daily urine output was reduced from around 8 to 3 liters per day with a corresponding decrease in the fluid intake.

Both subjects received a constant diet during the period of observations and for several days preceding. The data in Fig. 1 assumes a constant excretion in the stool of the various elements each day. Although we know that this is not absolutely true we have data which shows that the variations are such as to have no influence upon the picture presented here. The urinary excretion in these cases is therefore a good measure of the mineral balance. In the normal it happens that the chlorine does not follow the sodium as closely as it might because this subject had 5 gm. of KCl added to his diet each day and the percentage variation in chloride excretion is consequently less than in sodium excretion, with the same absolute change in both. Unlike the normal the sodium balance does not become negative in diabetes insipidus as a result of "pituitrin" therapy. In this patient it happens that it actually becomes positive, at least for 4 days and although not a constant result in this condition it is frequently the case. The potassium balance is positive in both the normal and in this and other patients.

It is possible that the difference in the effect of "Pituitrin" on the sodium balance in normals and diabetes insipidus is an accidental result of the difference in the effect of the drug on the urine volume. If it were possible for a patient with diabetes insipidus to receive a normal fluid intake with ensuing normal urine volumes and the normal to be given an excessive amount of fluid until the administration of "pituitrin" when both would get ordinary quantities of fluid it is conceivable that the influence on the sodium balance might be reversed. It is planned to examine this question at the earliest opportunity.

8523 C

Diurnal Variation in Excretion Rate of Formed Elements in Urine in Hemorrhagic Bright's Disease.

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The enumeration and differential counting of the formed elements in the urine forms the basis of the most useful clinical methods for distinguishing the different types of Bright's Disease[1] and yields valuable information concerning prognosis.[2] The usual method consists in examining a fairly concentrated specimen of urine collected over 9 or 12 hours during the night.[1] Frequently it becomes desirable to examine specimens of urine over a shorter period and in the daytime, often without any particular precautions of diet. To this end the diurnal variation in the rate of excretion of the formed elements in the urine was made in 2 patients with hemorrhagic Bright's Disease. Two observations were made upon each of these subjects. They were both young adult males (case 1 was 28 years old and case 2, 20 years old). The specific gravity of the specimens (1.012-1.018) was frequently not so high as is desirable[1, 2] but this was compensated for in large degree by the prompt examination of the specimens shortly after they were voided. The formed elements were counted by the method devised by Addis.[1] Urine was collected over 2-hour periods from 6 A.M. to 10 P.M. and then the 8 hours night urine collected in a single specimen. In the first experiment on both patients they remained in bed all day while in the second experiment they were up and about during the day. The second experiments were made 5 days (case 1) and 2 days (case 2) respectively after the first ones. Both patients had a mild hypertension but no anemia or nitrogen retention. The renal function in case 1 was 60% and in case 2 about 45% of normal. Both patients were recovering from the initial stage[1] of hemorrhagic Bright's Disease.

The results in Fig. 1 (the significance of the various lines on the charts is indicated by the legends used in the ordinate scales, *e. g.*, the heavy continuous line is urine volume rate, etc.) show a considerable degree of uniformity in the several experiments. The greatest rate of urine excretion is always over the lunch period be-

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[1] Addis, T., *J. Am. Med. Assn.*, 1925, **85**, 163.

[2] Addis, T., and Oliver, J., The Renal Lesion in Bright's Disease, P. B. Hoeber, Inc., New York, 1931, p. 9.

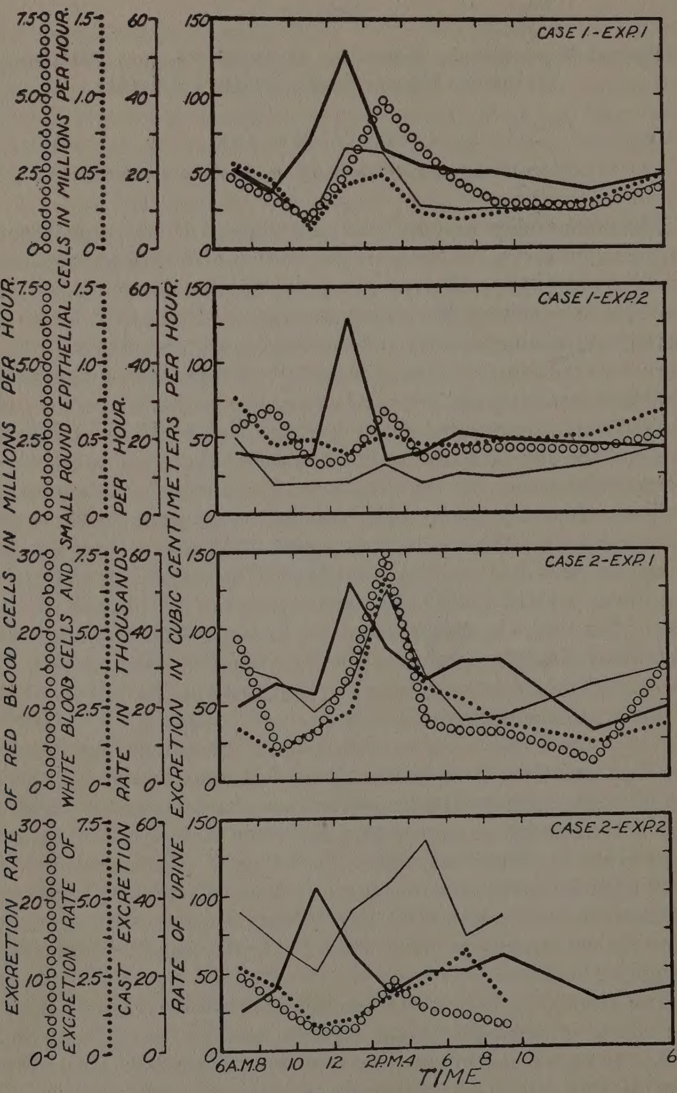

FIG. 1.

tween 10 and 12 A.M. The number of red blood cells excreted tends to be the highest and in all cases shows a peak of variable height during the 2 to 4 P.M. period. There is no available explanation for this. It bears no relation to the diurnal variation in renal function.[3] The variations in the rate of excretion of leucocytes and epithelial cells (these are enumerated together because indistinguishable[1]) and to a lesser degree of casts (in both cases all the casts happened to be hyaline) were in general similar to those of the erythrocytes. The rate of excretion during the morning tended to be lower than at night followed by the mid-afternoon rise. During the late afternoon and evening as well as the night (with only an average observation between 10 P.M. and 6 A.M. we must guess at the latter) the rate of excretion of formed elements appears to be at a fairly steady level. As a practical point this would lend weight to the current practice of measuring the excretion rate of formed elements on the night urine whenever feasible quite apart from the fact that it is easier to obtain the desired urine concentration and acidity during the night with the least inconvenience to the patient. It should also be pointed out that from the experiments reported here it is obvious that the rate of excretion of the formed elements at any time of day if properly carried out would generally lead to the same conclusions about the patient for in spite of the considerable variations the number excreted tend to remain of the same order of magnitude.

8524 P

Measurement of Insulin Action.*

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The insulin required to maintain a normal blood sugar level in depancreatized dogs has been studied by Holm[1] and in the laboratories of Houssay[2] and Soskin.[3, 4] Holm and Houssay found that 0.01 unit insulin per kilogram of body weight per hour was necessary. Soskin found values as low as 1-175th unit insulin per kg. per hour in dogs under pento-barbital sodium (Abbott).

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[3] MacKay, E. M., *J. Clin. Invest.*, 1928, **6**, 505.

* We wish to express our sincere appreciation for the generous gifts of insulin and amytal from the Eli Lilly Co., and for nembutal from the Abbott Laboratories.

[1] Holm, *Arch. f. exp. Pathol. u. Pharmakol.*, 1927, **121**, 368.

[2] Houssay, B. A., Lewis, J. T., Foglia, V. G., *Compt. Rend. Soc. Biol.*, 1929, **101**, 241.

[3] Soskin, S., Allweiss, M. D., *Am. J. Physiol.*, 1934, **110**, 4.

[4] Soskin, S., Allweiss, M. D., Cohn, D. J., *Am. J. Physiol.*, 1934, **109**, 155.

We have determined the insulin requirement in dogs of different sizes which have completely recovered from the pancreatectomy. These dogs were maintained at a constant weight on a measured balanced diet and sufficient insulin to keep the urine nearly sugar-free. They received no food or insulin for 24 hours prior to a determination. No anesthetic was used. The initial high blood sugar was lowered to about 100 mg.% by an intravenous injection of 2-6 units of insulin, depending upon the size of the animal. A continuous or hourly intravenous injection of a weak solution of insulin in physiological saline was started about 4-6 hours following the initial injection. An amount is given which will keep the blood sugar at about 120 mg. %. Table I summarizes the results on 4 different animals.

TABLE I.

Dog T. 9.5 kg. Depancreatized 3-7-35		Dog B. 9.5 kg. Depancreatized 12-5-35		Dog S. 15 kg. Depancreatized 9-27-35		Dog Bl. 15 kg. Depancreatized 10-24-35	
Date	Units/-K.Hr.	Date	Units/-K.Hr.	Date	Units/-K.Hr.	Date	Units/-K.Hr.
1935:		1936:		1935:		1935:	
10-30	.0095	1-16	.0094	11-9	.0126	11-15	.014
11-5	.0102			11-16	.013		
11-13	.0105			11-23	.014		
12-4	.0094			11-29	.014		
1936:							
1-4	.0094			12-6	.014		

Determinations of the insulin-glucose ratio were made. In one experiment the basal insulin requirement was increased from 0.21 unit insulin per hour to 0.41 unit and a continuous intravenous injection of 5% glucose was given. After 5 hours it was possible to inject 3.48 gm. of glucose per hour with the blood sugar constant at 125 mg. %. On the basis of the added insulin, the insulin-glucose ratio was therefore 1 unit to 17.4 gm. In another experiment 1 unit additional insulin per hour was added to the basal rate of 0.09 units insulin per hour, making a total of 1.09 units of insulin per hour. Eleven hours after starting the injection of glucose, 9.0 gm. per hour were being given with a blood sugar of 116 mg. %. More experiments of this nature are in progress with varying amounts of insulin.

Amytal and nembutal both apparently cause a marked lowering of blood sugar if given during the injection of the glucose and insulin. A 15 kg. dog received 4.0 gm. of glucose intravenously and 1.21 units of insulin per hour. Following the intravenous injection of 400 mg. of sodium amytal, the blood sugar fell from 150 mg. % to 94 mg. % in 30 minutes. A rapid increase in the injection rate of glucose was possible, 12 gm. per hour being given 2 hours later with a blood sugar of 130 mg. %. In another experiment on the same dog insulin was given at the rate of 1.21 units, and 1.9 gm. of glucose per hour. In 20 minutes after the intravenous injection of 2 cc. of nembutal, the blood sugar had fallen from 140 mg. % to 110 and in 50 minutes to 88 mg. %. A fall of 40 mg. % in blood sugar in 30 minutes has also been demonstrated after nembutal in one of the 9.5 kg. dogs.

8525 C

Urea as a Solvent in Preparation of Antigen Extracts.

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The development of the study of allergy in recent years, and particularly the search for the etiologic factors, has focused attention upon numerous protein containing substances. These include foods, pollens, molds, epidermal structures, orris root, house dust and such less common factors as the scales on sand flies or the stinging organ of bees, wasps, etc. The substance suspected may be tested on the patient in various ways: (a) by direct application of it to the skin, to the conjunctivae, or if a food, it may be ingested; (b) the juice may be expressed from certain of the substances and used by application to a scratch on the skin, or by intracutaneous injection. This method is naturally limited in its application. The ideal method is one which would concentrate the active substance, change its physical or chemical composition little or none, and one which could be used on all types of substances. By analogy with immunologic phenomena, and in view of the evidence which has accumulated in the field of allergy, one is led to believe that the active allergic agent is protein or closely attached thereto.

Proteins in general are rarely water soluble to any extent; this is especially true after they have undergone the procedures necessary to separate them from the various substances mentioned above. The simplest extraction methods employ a solution of NaCl in various concentrations. Alkalies, notably a bicarbonate or hydroxide, or a neutral phosphate buffer solution, find most general use. Recently a glucose extract has been advocated. The multiplicity of methods indicates that none is entirely satisfactory. This fact led to the present attempt to utilize the peculiar solvent action of strong urea solution in the preparation of these and other active extracts.

Spiro[1] was the first to describe the solvent action for proteins of strong urea solutions. Ramsden[2] studied this property in more detail. Although a saturated or strong urea solution is necessary for rapid solution the preparation may then be greatly diluted. Urea is a neutral, relatively inactive and natural compound in the organism, well suited for injection. It is true[3] that it denatures proteins. Whether this denaturation is of the same type as suffered by alkali-treated proteins is not known. The relative influence of various denaturing agents upon antigens has not been carefully examined. Acetone[5] and heat[4, 5] denatured serum albumins are grossly altered immunologically. We hope to examine the immunological changes induced in proteins by solution in strong urea.

The urea extracts were prepared by triturating the original desiccated and powdered product or a dried simple extract of it with urea crystals and water sufficient to make a 50% urea solution. Five grams of powder were extracted with 50 or 100 cc. of the strong urea solution. Sterilization was effected by filtration through a bacteria-restraining filter, with some difficulty in the case of several of the extracts. It is probable that the bactericidal effect of strong urea solutions[6] may make this further treatment unnecessary.

Many protein extracts form, at refrigerator temperature, an irreversible precipitate, or they will do so when sufficiently diluted with a neutral isotonic solution. The lower concentration is usually necessary in skin testing. Thus far urea extracts have failed to show such undesirable changes. Certain extracts give a high percentage of positive reactions which can rarely be correlated with clinical sensitivity. There is evidence that the urea solutions may be free from these.

Fifty-five patients, presenting allergy-like complaints, were tested by the scratch (1:20 dilution) or intracutaneous (1:1,000 dilution) method, with a urea extract of several foods,* chicken feathers and a false ragweed pollen. The same patients were tested with conventional stock allergens—foods, pollens, epidermals, etc. Fortythree patients (78%) reacted to no stock allergen and to none of the urea extracts. Seven patients reacted to stock allergens, but to none of the urea solutions, and these reactions did not include a sensitivity to any of the foods, etc., used in making urea extracts.

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[1] Spiro, *Z. f. Physiol. Chem.*, **30**.

[2] Ramsden, W., *J. Physiol.*, 1902, **28**, XXXIII.

[3] Hopkins, F. G., *Nature*, 1930, **126**, 329, 383.

[4] Obermayer, F., and Pick, E. P., *Wein. Klin. Woch.*, 1906, **19**, 327.

[5] Miller, B. F., *J. Exp. Med.*, 1933, **58**, 625.

[6] Foulger, J. H., and Foshay, L., *J. Lab. and Clin. Med.*, 1935, **20**, 1113.

* Bean (lima), beef, celery, flaxseed, lamb, potato, rye and tomato.

These results are still consistent with specificity of the new extracts. Five patients (9.1% of 55) reacted to one or more of these extracts and, as indicated, also to stock allergens. Nine reactions, or an average of approximately 2 per reacting patient, were obtained. But 5 urea extracts† (½ of those employed) demonstrated any sensitivity. It is manifestly unfair to emphasize a comparison of these reactions with those obtained to stock allergens on the same 5 patients. These latter reactions, however, are of value as presumptive evidence in favor of allergy. The clinical complaints emphasized by the 5 patients were referable to the respiratory tract. All reactions obtained are potential causes of such symptoms.

Summary and Conclusions. Protein constituents of foods, pollens, epidermals and many special substances are important factors in allergy. The extraction of these components is a necessary feature in their study. The ideal extractive would be one that is easy to use, is generally applicable to all substances, produces a potent concentrated extract, which will remain in solution, modifies the original protein little or none, and, most important of all, one which will yield extracts that are capable of detecting the maximum of clinical sensitivities. If these ideals are met it is probable that the present problem of non-specific skin reactions would become insignificant.

Urea solutions do modify the original protein constituent: the degree and direction of this denaturation is still being investigated. Such extracts are easy to make, permit of considerable concentration; have found general application and seem to give few, if any, reactions on non-allergic individuals as judged by a series of 55 patients tested with 10 urea extracts. In the total of 550 tests, but 9 reactions (1.6%) were obtained and these were given by 5 of the 10 extracts. The 5 patients giving these were potential allergic patients, as their clinical conditions belonged to that group and each patient gave one or more reactions to stock allergens. There was absolute agreement—all negative—between the special and stock extracts on 43 patients. Further study by different workers is necessary before the method should be urged in substitution for the conventional ones now in widespread use.

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† Reactions were obtained to bean, celery, rye, tomato, chicken feathers and false ragweed.

8526 C

Relation of Anterior Pituitary to the Volume of Islet Tissue in the Male Rat.*

Boris Krichesky. (Introduced by B. M. Allen.)

From the Department of Zoology, University of California at Los Angeles.

The importance of the pituitary gland in carbohydrate metabolism has been generally recognized since the publication of the work of Houssay and his associates.[1] They reported that the removal of the hypophysis in toads and dogs considerably ameliorated the hyperglycemia which followed pancreatectomy, and their results have been amply confirmed by Barnes and Regan,[2] by Lucke,[3] and by others. The antagonistic action of the hormone from both the anterior pituitary and the islets of Langerhans on blood sugar levels is well known. The former tends to increase while the latter decreases blood sugar levels. The determination of morphological relationships, if any, existing between these 2 endocrine glands was thought to be of interest.

In this preliminary study, a comparison was made of the ratio of the total volume of islet tissue in the pancreas to the body weight. The findings from 23 male rats are reported here. These were divided into 3 groups. (1) 8 normal controls, (2) 8 hypophysectomized and (3) 7 hypophysectomized pituitary-injected animals. In the last group, injections were begun on the day of operation and were administered 4 times weekly in doses of 0.5 to 1.0 cc. The material used for injection was prepared by the method of Evans and Cornish and a few animals were treated with Phyone obtained from the Wilson Laboratories. All animals of groups 2 and 3 were hypophysectomized from 30 to 60 days before they were sacrificed. Identical conditions for all 3 groups were maintained during the course of the experiment, with a surplus of a standard diet always available. At the end of the experiment, the animals were killed, the entire pancreas removed, fixed in Bouin's solution and serially sectioned at 25 micra. Camera lucida tracings were made of all island tissue in every 10th section, the areas determined

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* Aided by a research grant from the University of California, and executed under the supervision of Dr. B. M. Allen.

[1] Houssay, B. A., and Biasotti, A., *Endocrinology*, 1931, **15**, 511; Houssay, B. A., Biasotti, A., and Rietti, C. T., *Compt. rend. Soc. de biol.*, 1932, **111**, 479.

[2] Barnes, B. O., and Regan, J. F., *Endocrinology*, 1933, **17**, 522.

[3] Lucke, H., Heydemann, E. R., and Hechler, R.. *Verhandl. d. deutsch. Gesellch., F. inn. Med.*, 1933, **45**, 164.

TABLE I.

Table Showing Ratio of Volume of Island Tissue to Body Weight in Normal, Hypophysectomized and Hypophysectomized Pituitary Injected Male Rats.

	Animal No.	Body Wt. gm.	Vol. of Islands cmm.	Ratio Vol. Islands / Body Wt.	Aver. Ratio
Group I	505	208	5.57	.027	
Normal Controls	507	140	5.31	.038	
	508	174	10.28	.059	
	510	156	7.11	.045	.038
	511	223	7.95	.036	
	513	224	8.91	.040	
	514	219	7.45	.034	
	515	197	6.44	.033	
Group II	501	184	11.09	.060	
Hypophysectomized	502	152	9.63	.063	
	503	125	8.59	.069	
	504	220	11.65	.053	.062
	506	171	9.86	.058	
	509	192	11.82	.061	
	512	141	7.72	.055	
	516	154	11.60	.075	
Group III	517	188	9.51	.051	
Hypophysectomized	518	183	9.35	.051	
Pituitary Injected	519	191	8.31	.043	
	536	201	9.86	.049	.051
	537	159	8.28	.052	
	539	211	10.03	.047	
	540	188	12.04	.064	

with a planimeter and the volumes calculated. Table I indicates the ratio of the volume of island tissue to the body weight in these three groups of animals.

From the data given, it can be seen that the ratio of the volume of island tissue to the body weight in the hypophysectomized animals has increased approximately 63% over that of the normal controls. In the group of hypophysectomized animals receiving 4 injections weekly of a crude alkaline extract of the pituitary beginning on the day of operation (group 3), the ratio is only approximately 34% greater than that of the normal controls. In this last group, it is apparent that administration of anterior pituitary substance inhibited, to a limited degree, the hypertrophy of island tissue that occurred on pituitary removal. These results are not in accord with the conclusions of Koster[4] who found that, although the pancreas of hypophysectomized dogs is usually atrophic, no specific changes occurred in the islands of Langerhans that would indicate a regulating influence of the hypophysis on the hormonal activity of the pancreas.

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[4] Koster, S., *Arch. f. d. ges. Physiol.*, 1930, **224**, 212.

Pacific Coast Section

Stanford University Medical School, February 5, 1936.

8527 C

Losses of N and C in Drying Feces of Cattle.

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In respiration trials for the determination of the N and C balances errors may result from losses of N and C during the drying of feces for analysis.

The N losses can be avoided by determining the N in fresh instead of dry samples of the excreta. The N determination in fresh samples involves more work than the determination in a composite sample of the dried feces. This extra work is, however, justified since the N losses during drying are considerable.

In order to measure the N losses by drying, aliquot samples of the feces, that were removed daily from the respiration chamber, were immediately mixed with concentrated H_2SO_4 in Kjeldahl flasks. The digestion was either made at once or the flask was closed until the next day's sample was added to it. Aliquot parts from the same feces that had been analyzed fresh were dried at 100°C. The N determination was then carried out with a part of the composite dry sample. The official Kjeldahl-Gunning-Arnold method[1] was used for the N determinations.

The difference in the N content of the total feces of a 10-days' respiration trial calculated from the analyses of the fresh samples and the N content calculated from the analysis of the composite dried sample was considered the N loss by drying and expressed in per cent of the N content as determined in the fresh samples. For 12 respiration trials with beef heifers this N loss amounted to $7.19 \pm 0.49\%$. In a sample taken immediately after defecation only 4.48% of the N was lost by drying. A day's storage in the feces collector of the respiration chamber and possibly a slight contamination of the feces with urine seems thus to increase the N loss during subsequent drying.

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[1] Assn. of Official Agricultural Chemists, Methods of Analysis, 1931, p. 21.

The wet combustion of feces samples with dichromate could be employed for the determination of the C in feces in a procedure similar to that used for the N determination. The dichromate method of C determination did, however, not prove to be as reliable for the C determination in feces as the combustion of a dry sample in the calorimetric bomb under 25 atmospheres oxygen pressure and subsequent absorption of the formed CO_2. We therefore determine the C content of the feces in a dry sample but measure the C loss during the preceding drying process in the apparatus sketched in Fig. 1.

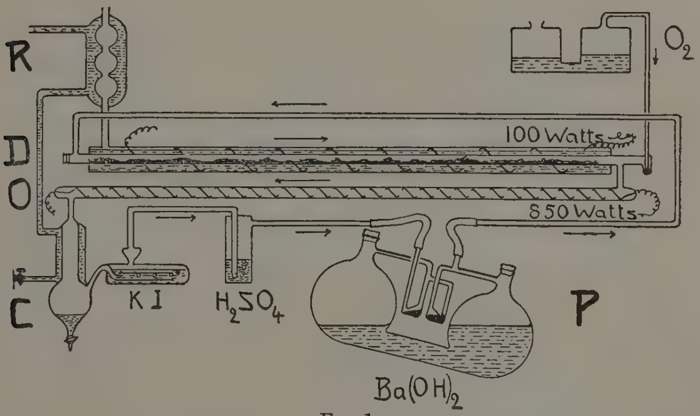

FIG. 1.
Apparatus for drying samples of feces and for determining the loss of CO_2 and other volatile C compounds during drying.

An aliquot part (½ to 1%) of the daily feces is spread into a Monel metal boat of approximately 1 cm. diameter and 1 m. length. This boat is placed in a tube of pyrex glass (D in Fig. 1) which is heated by water contained in an outer jacket. That water is kept boiling by an electric current of 100 Watts and condensed in the reflux cooler R. The drying tube D is connected to a silica combustion tube O that contains 20 gauge B and S Nichrome wire kept at dull red glow by an electric current of 850 Watts. The combustion tube is connected to a condenser C and this condenser to a wash bottle with neutral KI solution approximately 0.001 normal. Another washbottle connected to the first one contains concentrated H_2SO_4. A pair of connected 500 cc. bottles P containing 0.2 n $Ba(OH)_2$ solution completes the system. The bottle pair with the

Ba $(OH)_2$ solution is rocked (6 cycles per minute) and therefore acts as an air pump. A set of mercury valves as shown on Fig. 1 causes the air to flow in one direction only, as the battery rocks back and forth, so that a pulsating direct air current circulates in the system as indicated by the arrows in Fig. 1.

Function. The air from the drying tube D saturated with water-vapor passes the combustion tube O where any volatile C compounds evaporated during the drying process are oxydized to CO_2. The water-vapor is condensed in the cooler C. From there the air bubbles through the KI solution in order to absorb a volatile oxydizing agent which has been found to bleach the indicator* in the Ba $(OH)_2$ solution. After bubbling through the KI solution the air is again dried by concentrated H_2SO_4, then the CO_2 is absorbed in the Ba $(OH)_2$ solution of the rocking battery and the CO_2-free air flows to the drying tube D, completing its cycle. The oxydation of volatile C compounds causes a shortage of O_2, the drying tube is therefore connected to an O_2 supply (Fig. 1) from which O_2 is sucked into the system whenever a negative pressure develops.

As a result of the high rate of rotation of air in the system the drying process of the feces requires only approximately 3 hours. The amount of CO_2 absorbed in the Ba $(OH)_2$ solution is determined by titration,[2] and indicates the amount of C lost from the feces sample during drying.

The mean loss of C by drying the feces from 14 respiration trials (comprising 140 days of experiment) amounted to 1.36 ± 0.08% of the C content of the feces.

Drying experiments carried out without combustion of the volatile C compound indicated that 35 to 55% of the C lost from the feces by drying are given off as preformed CO_2, the rest as volatile combustible C compounds.

The method of drying aliquot parts of feces and determining the losses of C as described here has been found satisfactory and has been incorporated in the regular procedure of conducting respiration experiments at our institute.[3]

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* 1 part Cresol red-Na 0.1% in water.
3 parts Thymol blue-Na 0.1% in water.
Kolthoff, Die Mass analyse, p. 63, 1928.

[2] Kleiber, M., The California Apparatus for Respiration Trials with Large Animals, Hilgardia 9, p. 38 ff., 1935.

[3] See (2), p. 48.

8528 P

Effect of Cholesterol Feeding on Growth of Rats.

RUTH OKEY, HELEN L. GILLUM AND LOIS STEWART GODFREY.*

From the Laboratory of Household Science, University of California, Berkeley.

In this laboratory, during the past few years, we have fed to a total of several hundred young rats diets containing 1% cholesterol. We have found, almost without exception, that if the basic diet to which the cholesterol was added was adequate to support entirely normal growth on a long-time basis, there was no significant difference between the growth curves of the cholesterol-fed animals and those of the littermate controls which received the same diets without the added cholesterol. Moreover, this was true as well for certain vitamin-deficient diets, notably those lacking A.

This finding is entirely at variance with that reported by Sperry and Stoyanoff[1] for synthetic diets. This would seem to indicate the value of analysis of the diets used in the two laboratories with this point in view.

The composition of our diets has already been reported.[2] Extracted casein was used as a source of protein in both laboratories and at levels sufficiently nearly alike as to probably rule out differences in behavior due to protein intake. Sperry, *et al.*, have used sucrose, and we have used starch as a source of carbohydrate. While the cornstarch used in our laboratory certainly did not contain enough betaine, as Best and Ridout[3] have suggested that certain samples of potato starch may, to largely prevent cholesterol ester deposition in the livers of our rats, nevertheless there is no proof that it did not contain some factor which may be necessary for the growth of cholesterol-fed rats.

Again, we have used in this laboratory less highly purified sources of vitamins A, D, and, in some case, B and G, than the Columbia investigators. The behavior of vitamin-deficient animals fed cholesterol in this laboratory has been such as to indicate that lack of none of the vitamins just mentioned is in itself responsible for the retarded growth of Sperry's cholesterol-fed rats, but it does

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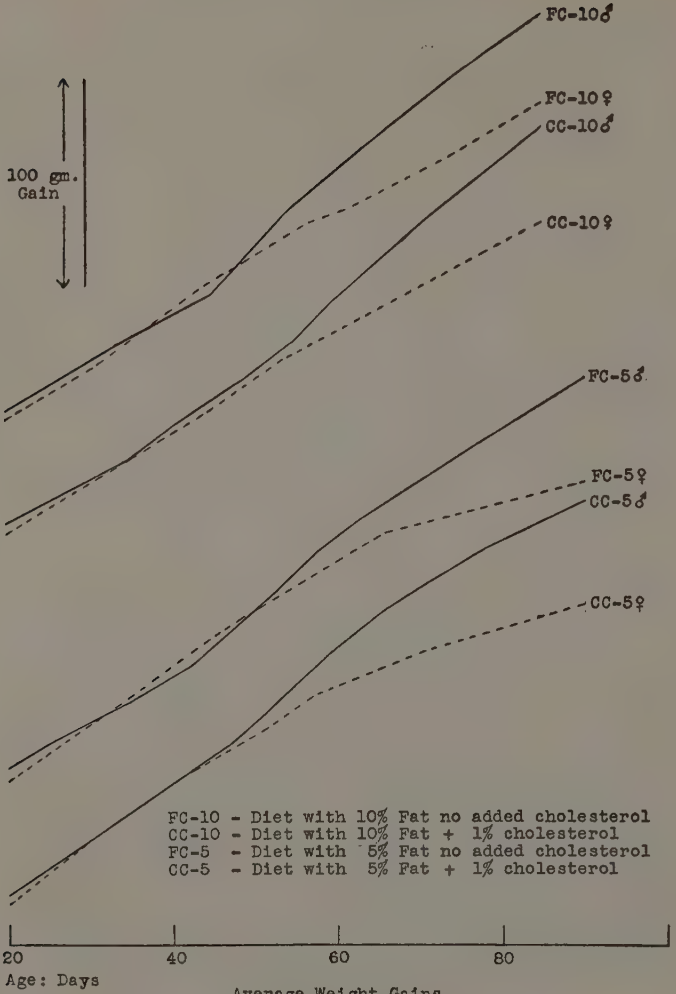
* Deceased, November 16, 1935.

[1] Sperry and Stoyanoff, *J. Nutr.*, 1935, **9**, 131.

[2] Okey, Gillum and Yokela, *J. Biol. Chem.*, 1934, **107**, 207, and Okey, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1003.

[3] Best and Ridout, *J. Physiol.*, 1935, **84**, 7P.



Average Weight Gains
Groups of 12 Rats

not eliminate the possibility that some other accessory factor associated with our vitamin sources may have made it possible for our cholesterol-fed rats to grow normally.

Rats fed cholesterol in this laboratory have, when the fat intake was as high as that used by Sperry, shown liver cholesterol ester levels within the same range. The larger proportion of our rats have, unfortunately for the sake of comparison, been fed cholesterol at lower levels of fat intake and have consequently had a lower percentage of total liver lipid. Analysis of all of our figures at present available shows little or no relationship between level of liver cholesterol ester and growth rate. Data at present available do not justify any conclusion as to the exact nature of the factor responsible for this difference in results in the two laboratories. It seems probable that some accessory substance is necessary to permit normal growth coincident with the storage of high levels of cholesterol ester in the liver, and that lack of this, rather than the cholesterol itself, may have been responsible for the retarded growth reported.

8529 P

Prevention of Intranasally Inoculated Poliomyelitis in Monkeys by Previous Intranasal Irrigation with Chemical Agents.*

E. W. SCHULTZ AND L. P. GEBHARDT.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

That the normal portal of entrance of poliomyelitis virus is the olfactory nerve is now well established.[1] It is also clear from recent studies[2] that the administration of specific immune serum does not afford significant protection against subsequent intranasal instillation of monkeys with poliomyelitis virus. The relative ineffectiveness of immune serum may be attributed to the fact that the terminals of the olfactory nerve are so situated that they cannot be effectively guarded by immune plasma. Once established in a nerve the virus travels to its destination in the medulla and cord by axonal paths,[3] quite safely out of reach of immune substances in the plasma. Recent attempts at active immunization against this disease have also proved disappointing in that the injection of vaccines results merely

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* Studies supported by funds allotted by the President's Birthday Ball Commission for Infantile Paralysis Research.

[1] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728; Brodie, M., and Elvidge, A., *Science*, 1934, **79**, 235; Lennette, E. H., and Hudson, P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1444.

[2] Schultz, E. W., and Gebhardt, L. P., *J. Ped.*, 1935, **7**, 332.

[3] Faber, H. K., *Medicine*, 1933, **12**, 83; Faber, H. K., and Gebhardt, L. P., *J. Exp. Med.*, 1933, **57**, 933.

in the production of humoral antibodies, with no significant increase in resistance to virus administered by the intranasal route.[4] In the face of this impasse it becomes necessary to seek a practical solution of the poliomyelitis problem in other directions.

In May, 1935, shortly after launching our present studies, Armstrong and Harrison[5] reported protection in monkeys following intranasal instillation of a 4% solution of alum. Their observations have recently been confirmed and extended by Sabin, Olitsky and Cox,[6] who add that protection is also afforded by 4% tannic acid. We wish to report similar results with picric acid, p-nitrophenol, trinitrocresol and mercurochrome.

Our observations are based on a number of experiments involving more than 120 test monkeys and controls. We are permitted to present these observations in only sufficient detail to indicate the scope of the investigations and the trend of the results.

In 3 experiments in which a total of 11 monkeys were given intranasal irrigations with 1% picric acid on 3 successive days, 10 animals have without further treatment exhibited a well defined resistance to intranasal instillations of virus for periods ranging from at least 2 days to more than 69 days. Two animals resisted intranasal instillations of virus 1, 18 and 40 days after treatment; one resisted virus instillations 69 days after treatment; 3 out of 4 in one experiment, resisted instillations 7 and again 38 days after treatment, while one developed the disease following the first instillation of virus 7 days after the treatments. The remainder have not yet been tested for resistance beyond the second day following treatment. The results of several experiments in which only a single intranasal wash with 1%, 0.5, 0.25, 0.1 or 0.01% of picric acid preceded the instillations of virus have been inconstant and indicate that for fairly uniform results *repeated* washings with 1% picric acid are necessary.

Three daily intranasal washes with 1% p-nitrophenol, or with 1% trinitrocresol also seem effective in protecting monkeys against intranasal instillations of virus for at least 2 days, while 1% ammonium picrate seems to be without any protective action.

In an experiment in which 3 monkeys were given intranasal irrigations with 1% mercurochrome on 3 successive days, all resisted instillations of virus one day and again 18 days later. Three additional monkeys so treated resisted virus instillations 2, 31 and 57 days later. Further instillations to determine the duration of the resistance remain to be made.

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[4] Schultz, E. W., and Gebhardt, L. P., *Calif. and Western Med.*, 1935, **43**, 11; Olitsky, P. K., and Cox, H. R., *J. Exp. Med.*, 1936, **63**, 109.

[5] Armstrong, C., and Harrison, W. T., *Pub. Health Rep.*, 1935, **50**, 725.

[6] Sabin, A. B., Olitsky, P. K., and Cox, H. R., *Proc. Soc. Am. Bact., J. Bact.*, 1936, **31**, 35.

In all of the virus instillations referred to above the controls generally equalled the animals treated with a given reagent and the percentage of infection in the parallel control group averaged above 90%. The intranasal irrigations with the chemical solutions indicated were all carried out thoroughly while the animals were under ether anesthesia and held in a special vertical holder, with the head downward.

Further studies are in progress not only with the reagents above mentioned, but with a number of others, including a variety of dye stuffs, chosen particularly for their potential ability to modify the permeability of the normal portal of entrance of this virus. Among the various possibly effective agents may be one or more which may prove effective, agreeable, and harmless enough for prophylactic application in man during epidemic periods.

8530 C

Acute Toxicities of Rotenone and Mixed Pyrethrins in Mammals.

MICHAEL B. SHIMKIN AND HAMILTON H. ANDERSON.

From the Pharmacological Laboratory, University of California Medical School, San Francisco.

Rotenone and pyrethrum are proposed as harmless substitutes for inorganic insecticides,[1] and mixed pyrethrins are recommended as anthelmintics[2] and as scabicides.[3] The only critical report on the toxicity of rotenone in mammals is by Haag,[4] who indicates that there is little if any danger of acute intoxication following the ingestion of foodstuffs sprayed with rotenone. Chevalier[2] states that pyrethrum orally produces no untoward effects even when given to children.

In order to ascertain the probable toxic range of rotenone* or mixed pyrethrins† after absorption into the body, these drugs were given intraperitoneally or orally to guinea pigs, rats, and mice in 0.1-14% dilutions in ethylene glycol or petroleum oil, respectively, Animals were observed for one month. Controls, given the solvents alone, were unaffected over the period of observation.

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[1] Roark, R. C., *Ind. Eng. Chem.*, 1935, **27**, 530.

[2] Chevalier, J., *Bull. l'acad. de Med.*, 1928, **99**, 446.

[3] Sweitzer, S. E., and Tedder, J. W., *Minn. Med.*, 1935, **18**, 793.

[4] Haag, H. B., *J. Pharmacol. Exp. Therap.*, 1931, **43**, 193.

* Kindly supplied by Dr. R. C. Roark, Insecticide Division, Department of Agriculture, Washington, D. C.

1. Rotenone injected intraperitoneally killed half or more of the animals in doses of 2 to 10 mg. per kg. (Table I). Death occurred within 30 minutes in 50% of the fatalities, and within 14 days in the remainder. Toxic symptoms noted were: respiratory depression, incoordination, clonic convulsions, muscle tremors, and death due to respiratory failure. At necropsy one-third of the dead animals showed pulmonary congestion; no peritonitis or ulceration at the site of the injection were encountered.

Orally, the drug was given in powder form in gelatine capsules to guinea pigs, and in a 1% dilution in ethylene glycol by rubber tube to lightly anesthetized rats. The average minimal killing dose was 75 to 100 mg. per kg., and, as on intraperitoneal injection, was more toxic for rats than for guinea pigs (Table I). Thirty per cent of the fatalities occurred in 4 to 24 hours, 50% in 24 to 48 hours, and the rest in 3 to 10 days. Seventy per cent of the dead animals showed pulmonary congestion of varying degrees, and in 40% the mucosa of the stomach was reddened or sloughed. The animals surviving lost up to 10% of their initial weight in 3 days, but gradually regained it.

One per cent rotenone placed into a rabbit's eye produced immediate irritation and a transitory reddening of the conjunctiva. The solvent caused no effect.

TABLE I.
Acute Toxicity of Rotenone, in Ethylene Glycol.

	Dose in mg./kg.	Ratio of deaths to number of animals used		
		Mice	Rats	Guinea Pigs
Intraperitoneal:				
	1		0/9	
	2	0/10	11/14	0/5
	3		4/5	
	5	8/10	10/10	0/10
	10	9/10		5/15
	15			9/10
	20			4/5
Oral:				
	50		2/10	
	75		6/10	0/8
	100		8/10	4/10
	200		10/10	8/10

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† Kindly supplied by Dr. C. B. Gnadinger, McLaughlin-Gormley-King Co., Minneapolis, Minnesota.

Investigation of the chronic toxicity of rotenone given orally has shown that only one of 8 guinea pigs died after receiving 4 doses of 25 mg. per kg. in 10 days.

2. The mixed pyrethrins were used in 0.1 to 2.4% dilutions in petroleum oil, or as the oleoresin, containing 14% of pyrethrins I and II according to Gnadinger.[5] Injected intraperitoneally, the mixed pyrethrins killed at 100 to 150 mg. per kg. (Table II). The symptoms of toxicity noted were: increased respiratory rate, hyperexcitability, incoordination, tremors and paralysis, and death due to respiratory failure. Hyperexcitability at times continued for over 24 hours. One-third of the animals dying succumbed in 10 to 24 hours, one-third in 3 to 5 days, and the rest within 22 days. A small number of animals died almost immediately after injection of small amounts of the substance. Sixty per cent of the necropsied animals showed pulmonary congestion, and 10% had adhesive peritonitis.

TABLE II.
Acute Toxicity of Mixed Pyrethrins, in Petroleum Oil.

	Dose in mg./kg.	Ratio of deaths to number of animals used		
		Mice	Rats	Guinea Pigs
Intraperitoneal:				
	25	2/10		
	50	3/10	1/10	2/10
	100	5/10	3/10	5/10
	150	6/10	9/15	6/10
	200	10/10	13/15	9/10
Oral:				
	500		0/10	
	1000		0/9	1/10
	1500		0/6	5/8

The oral doses were administered by rubber tube, to lightly anesthetized animals. One-half of the guinea pigs died at 1500 mg. per kg., while rats tolerated this dose. Larger amounts could not be given safely, *i. e.,* without rupturing the stomach. The guinea pigs died in 48 hours, and at necropsy two-thirds (including some killed later) had pulmonary congestion, about half had damaged gastric mucosa, and one showed peritonitis. The survivors lost 10-15% of their initial weight and appeared ill for several days. The drug solution caused a yellow discoloration in the anal region in 2 to 3 hours, in several, and diarrhea, lasting 24 hours, was present in half of the animals. Rats given large amounts of mixed pyrethrins showed hair loss and skin irritation around the anal region; this occurred less frequently in the guinea pigs.

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[5] Gnadinger, C. B., Pyrethrum Flowers, McLaughlin-Gormley King Co., Minnesota, 1933.

Mixed pyrethrins, 2.4% rubbed into the hair and skin of six animals, produced no hair loss or skin irritation in rats or guinea pigs. One per cent pyrethrum placed into a rabbit's eye produced no immediate irritation, but a conjunctival bleb formed in 6 hours.

Mixed pyrethrins given in single doses of 500 mg. per kg. and repeated 6 times in 2 weeks (total administration of 3.0 gm. per kg.) killed 2 out of 8 rats.

8531 C

Acute Intraperitoneal Toxicity of Some Plant Growth Substances for Mice.

H. H. ANDERSON, M. B. SHIMKIN AND C. D. LEAKE.

From the Pharmacological Laboratory, University of California Medical School, San Francisco.

Zimmerman and Wilcoxon[1] report the rooting and bending responses of plants to various "growth-promoting" compounds. Of the agents we studied, they find the per cent concentrations necessary for positive bending or injury to be (in order of decreasing toxicity): indolacetic acid 0.5; alpha-naphthalenacetic acid 1.5; indolproprionic acid 2.0; indolbutyric acid 2.0; and phenylacetic acid 3.0. In the human body the indole acids are derived presumably from tryptophane, and indolacetic acid can be recovered from urine. In carcinomata indolacetic acid occurs in concentration twice that of surrounding tissues according to Kögl, Haagen-Smit and Tönnis.[2]

We desired to determine whether any correlation exists between the relative toxicity of these compounds for plants and their relative toxicity in mammals. Mice, observed for 4 weeks, were used as test animals. The substances were dissolved in water or di-ethylene glycol, and fresh 1% dilutions were injected intraperitoneally. Indolacetic acid* kills half or more of the animals injected at 25 mg. per kg., alpha-naphthalenacetic acid† at 100 mg. per kg., indolproprionic and[‡] at 100 mg. per kg., indolbutyric acid[‡] at 100 mg. per kg., and phenylacetic acid[§] at 300 mg. per kg.

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[1] Zimmerman, P. W., and Wilcoxon, F., *Contrib. Boyce Thompson Institute*, 1935, **7**, 209.

[2] Kögl, F., Haagen-Smit, A. J., and Tönnis, B., *Z. Physiol. Chem.*, 1933, **220**, 162; Kögl, F., Haagen-Smit, A. J., and Erxleben, H., *Z. Physiol. Chem.*, 1933, **220**, 137.

Deaths occur in 10 to 20 minutes with large doses, and are delayed from 48 hours to 13 days with amounts just above tolerance. Respiratory embarrassment and failure were the only consistent toxic manifestations noted. Pulmonary congestion was seen in one-third of the animals examined at necropsy; gross pathologic changes other than pulmonic were not observed in animals dying within 48 hours. Solutions of indolacetic acid which were exposed to light and air for 7 days become amber-colored and are less toxic than fresh preparations, killing at 200 mg. per kg.

The lethal doses of the 5 compounds studied stand approximately in the same relative order for mice according to our findings as the toxic range noted for plants, on the basis of Zimmerman's and Wilcoxon's data. Table I summarizes our findings.

TABLE I.
Acute Intraperitoneal Toxicity in Mice of Some Plant Growth Substances.

Drug in 1% solution	Lethal dose in mg./kg.	Ratio of deaths to No. of animals used	Time of death
Indolacetic acid	25	12/19	6 hr. to 25 days
Indolacetic acid (after standing one week)	200	9/15	20 min. to 3 "
α-Naphthaleneacetic acid	100	5/10	20 " to 6 "
Indolproprionic acid	100	16/20	1 hr. to 10 "
Indolbutyric acid	100	8/15	24 " to 13 "
Phenylacetic acid	300	11/15	10 min. to 10 "

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[*] Generously supplied by Merck and Co., Rahway, N. J.

[†] Generously supplied by Dr. P. W. Zimmerman, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.

[‡] Obtained from Dr. R. H. Manske, National Research Council, Ottawa, Canada.

[§] Obtained from Eastman Kodak Company, Rochester, N. Y.

Illinois Section.

Northwestern University Medical School, February 18, 1936.

8532 P

Measurements of Bone Growth by Alizarine Injections.*

I. Schour.

From the Department of Dental Histology, University of Illinois College of Dentistry.

Sixteen white rats 3 months of age were given 2 intraperitoneal injections of 0.5 cc. of 2% Alizarine Red S (Sodium Sulphalizarate) of color index 1034 (Coleman and Bell Company) 4 or 6 days apart and killed 2 days after the last injection. The dosage ranged from 39 to 62 mg. per kg.

Microscopic examination of transverse ground sections of the mid-root level of the upper molar teeth showed a red line in the alveolar bone that was calcifying at the time of each injection.

The amount of bone laid down in 24 hours was obtained by measuring the vertical distance between the 2 red lines in μ and dividing this amount by the number of days intervening between the 2 injections. The measurements were made of fields in which the 2 red lines followed a parallel course for at least a short distance. Tangential planes were avoided.

A total of 471 measurements were made by M. M. Hoffman, M. Engel and the author, who found the average amount of alveolar bone growth per 24 hours to be 4(3.98), 4(4.04) and 4(3.99) μ respectively. The range was 1.62-6.60 μ. Sixty-eight per cent of the measurements ranged between 3.5 to 4.5 μ and 87% ranged between 3 and 5 μ per 24 hours (Fig. 1). It is interesting that the daily amount of alveolar bone apposition was found to be approximately one-fourth the amount of the daily apposition (16μ) of enamel and dentin in the incisor of the rat.[1]

The alveolar bone was found to be a favorable site for these

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* This investigation was aided in part by a grant from the Graduate School Research Board of the University of Illinois.

[1] Schour, I., and Steadman, S. R., *Anat. Rec.*, 1935, **63**, 325.

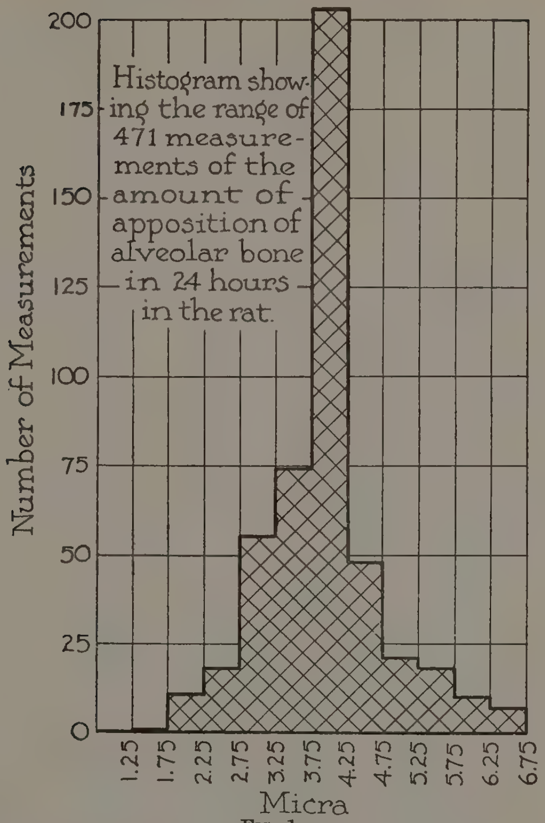

FIG. 1.

measurements because it grows continuously throughout life in its adjustment to the microscopic and physiologic movement of the teeth.

Multiple injections of sodium sulphalizarate present advantages over feeding of madder or alizarine and offer a prompt and accurate method of measuring the rate of apposition of bone in normal and experimental conditions.

Experiments are being continued in which this method is applied for the purpose of measuring the amount of daily apposition of enamel, dentin and bone in higher animals.

8533 C

Gastric Mucin a Prophylactic Against Gastro-Duodenal Ulcers and "Acute" Toxicity Resulting from Cincophen.

P. E. Reid and A. C. Ivy.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

It has been shown by Van Wagoner and Churchill[1] and Barbour and Fisk[2] that relatively large doses of cincophen administered daily by stomach tube will cause gastro-duodenal ulcer and liver damage. The occurrence of ulcer after cincophen administration has recently been reported to occur in man.[3] It has also been found that gastric mucin is prophylactic against the gastro-duodenal ulcer and disturbance of nutrition that results in some biliary fistula dogs[4] and is of benefit in the nutritional disturbance that occurs in some Eck fistula (liver damage) dogs.[5] Accordingly, we decided to ascertain if gastric mucin might prevent the occurrence of "peptic" ulcer in and prolong the life of dogs receiving large doses of cincophen.

Twenty-eight healthy dogs were used. Fifteen served as controls in that they received only cincophen, and 13 received both cincophen and mucin. The cincophen was made up in a starch paste solution (20 gm. of cincophen, 40 gm. starch and 2000 cc. H_2O), and administered by stomach tube to all dogs once daily in a dose of 100 mg. per kilo. The dogs that were given mucin received 30 gm. in solution in water about 2 hours before the cincophen in the morning and a second dose of 30 gm. during the evening. The dogs were given the regular stock diet consisting of yellow corn meal, bone soup, ground meat and baker's bread. The treatment was continued in the control group until they died, and in the group treated with mucin until they died or the experiment was terminated at 60 or 80 days. Portions of the liver and kidneys were removed for histologic study.

Results. Gastro-duodenal ulcer. Controls. Gastro-duodenal ulcers were found in all of the 15 control dogs in from 7 to 59 days. The ulcers were like those obtained by Van Wagoner and Churchill. In regard to susceptibility to the cincophen, the dogs varied considerably as is shown in Table I. The average length of life was 22.4 days.

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[1] Van Wagoner and Churchill, Proc. Soc. Exp. Biol. and Med., 1931, **28**, 581.

[2] Barbour and Fish, *J. Pharm. and Exp. Therap.*, 1933, **48**, 341.

[3] Block and Rosenberg, *Am. J. Dig. Dis. and Nutr.*, 1934, **1**, 29.

[4] Ivy and Kim, *J. Am. Med. Assn.*, 1931, **97**, 1511; Proc. Soc. Exp. Biol. and Med., 1932, **29**, 686.

[5] Crandall, Roberts and Gibbs, Proc. Soc. Exp. Biol. and Med., 1932, **29**, 1082.

TABLE I.
Dogs Receiving Cincophen Alone.
Cincophen: 100 mg. per kilo daily.

Dog No.	Initial Weight lbs.	Loss (—) or Gain (+) wt. lbs.	Length of life, days	Ulcer	Cause of Death
1	26.4	— 1.7	9	+	Hemorrhage from multiple gastric erosion and a large pyloric ulcer
2	39.1	— 0.5	7	+	Sacrificed due to asthenia. Gastric erosions and large pyloric ulcer
3	26.4	— 3.3	14	+	Perforating ulcer near pyloric outlet
4	21.8	— 0.9	7	+	Gastric erosions and large pyloric ulcer
5	24.0	— 3.2	19	+	Hemorrhage from ulcer at pyloric outlet
6	19.8	— 2.2	14	+	Hemorrhage from ulcer at pyloric outlet
7	17.6	— 5.3	11	+	Sacrificed. Ulcer of pylorus and duodenum
8	19.8	—	22	+	Pneumonia. Large ulcer at pyloric outlet
9	19.0	— 6.0	16	+	Perforation—duodenal ulcer
10	22.0	— 8.8	15	+	” ” ”
11	18.7	— 3.3	21	+	Ulcer at pylorus
12	35.6	—16.4	59	+	Duodenal ulcer—hemorrhage
13	27.0	— 8.6	26	+	Ulcer at pylorus
14	19.0	— 6.0	47	+	Perforation—duodenal ulcer
15	23.0	— 8.0	49	+	Duodenal ulcer
Aver. 15		— 5.3	22.4	15	

TABLE II.
Dogs Receiving Mucin and Cincophen.
Cincophen: 100 mg. per kilo daily. Mucin: 30 grams twice daily.

Dog No.	Initial Weight lbs.	Loss (—) or Gain (+) wt. lbs.	Length of Expt. Days	Ulcer	Cause of Death
1	16	+ 2.0	37	+	Perforated duodenal ulcer
2	16	+ 0.5	60	None	Sacrificed. Normal grossly
3	17	— 7.0	54	”	Pneumonia. Completely healed ulcer scar in duodenum
5	16	+ 2.5	60	”	Sacrificed. Normal grossly
6	39.7	—13.7	80	+	Sacrificed. Chronic indurated duodenal ulcer
7	36	— 6.0	80	None	Sacrificed. Normal grossly
8	26.5	— 5.0	80	”	” ” ”
9	35.5	— 3.0	80	”	” ” ”
10	20.0	+ 6.0	60	”	” ” ”
11	20.0	+ 1.0	60	”	” ” ”
12	22.0	— 0.5	60	”	” ” ”
Aver. 11 dogs		— 2.1		2 ulcers	18 % ulcers

Dogs 4 and 4b died of pneumonia on the 32nd day without ulcer. They are omitted from the table because they died prior to 60 days without ulcer.

In dog 3 pneumonia and in dog 6 hemorrhage accounts for some of the loss of weight.

Treated. The dogs receiving mucin and cincophen reacted quite differently as a group. All had some diarrhea during the first 2 weeks; one-half of them had a bloody diarrhea during this period. Five of them (Dogs 1, 3, 6, 7, 8) had some diarrhea and anorexia intermittently throughout the experimental period. The remainder after the first 2 weeks were apparently unaffected. Five even gained weight. Of the 11 dogs in which the experiment was completed, only 2 developed ulcer, or 18%, in contrast to the 100% incidence of ulcer in the control or untreated group. (Table II.)

The histology of the liver and kidney of both groups of animals is being studied by Dr. T. P. Churchill, Pathologist.

Conclusion. The administration of gastric mucin is markedly effective in preventing the gastro-duodenal ulcer and "acute" toxicity of cincophen in dogs.

8534 C

Dextrose Yield of Glycinin.

J. S. GRAY. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Since soy bean foods have been found to be useful in the diabetic dietary,[1] it was thought desirable to determine the dextrose conversion of soy bean protein.

Glycinin, a specific globulin, which constitutes 90-95% of the protein of the soy bean,[2] was prepared by a new method, since older methods were found to be tedious. Commercial soy bean flour was freed of fat by ether extraction. The residue was then suspended in water at a pH of 2-3, filtered, and the dissolved glycinin precipitated at its isoelectric point by adjusting the reaction to pH 4.7. The flocculent precipitate was washed with distilled water, redissolved at pH 2-3, filtered, and reprecipitated at pH 4.7. After washing, the precipitate was dried with alcohol and ether. This preparation contains 15.56% nitrogen, 5.5% moisture, 0.82% ash, and gives a negative Molisch test for carbohydrate.

Glucose yields were obtained on completely phlorizinized dogs, using the method applied by Janney[3] to the determination of glucose yields of other proteins. As a check on the method the yield of casein was also determined.

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[1] Friedenwald and Rurah, *J. Am. Med. Sci.*, 1910, **140**, 793.

[2] Osborne and Campbell, *J. Am. Chem. Soc.*, 1898, **20**, 419.

[3] Janney, *J. Biol. Chem.*, 1915, **20**, 321.

The average of 8 determinations of the glucose yield of glycinin, obtained on 5 dogs was 61%. The average of 11 determinations on 4 animals gave a glucose yield of 49% for casein, a figure which compares favorably with Janney's previous report of 48%.

TABLE I.

Protein	Total Known Amino Acids %	Total Glycogenic Amino Acids %	Glycogenic Amino Acids as % of Total %	Dextrose Derived from Glycogenic Amino Acids gm.	Dextrose as % of Total Amino Acid %	Exper. Yield %
Casein	95.1	52.8	55.6	36.8	39.1	49
Zein	103.4	58.0	56.5	41.2	43.9	53*
Gelatin	93.5	75.8	81.1	56.9	60.8	65*
Edestin	101.8	60.7	59.6	39.8	39.1	65*
Gliadin	99.0	72.9	73.6	48.1	50.0	80*

*As determined by Janney.[3]

It was found in the case of casein, zein, gelatin, edestin, and gliadin, proteins whose amino acid analyses are fairly complete, that there was a slight correlation between the experimental yield, and the percentage of sugar-forming amino acids in their molecules. A theoretical yield for each of these proteins was arrived at by calculating the total sugar-forming carbon in the glycogenic amino acids and converting this into terms of dextrose. This procedure gives the total amount of glucose which could be formed by the individual glycogenic amino acids. Theoretical yields calculated in this manner failed to show any correlation with the experimental yields. In every case they fell short of the experimental yields by amounts which were not related to the incompleteness of the protein analysis. This latter conclusion is based on the observation that by expressing the sugar produced by the amino acids as a percentage of the known amino acids the correlation with the experimental yields is not improved. There is no evidence to indicate whether this discrepancy is due to imperfect amino acid analyses or to the inadequacy of the phlorizinized dog as a subject for the determination of dextrose yields.

Conclusions. (1) The dextrose yield of glycinin is 61%; (2) confirming a previous report, that of casein is 49%; (3) the dextrose yield of a protein cannot be predicted by calculating the amount of dextrose which the component glycogenic amino acids are capable of forming in the animal organism.

8535 C

Determination of Reduced Ascorbic Acid in Small Amounts of Blood.

CHESTER J. FARMER AND ARTHUR F. ABT.

From the Departments of Chemistry and Pediatrics, Northwestern University Medical School, Chicago.

We previously presented data as to the quantity of reduced ascorbic acid in blood plasma and showed its dependence upon the dietary supply of vitamin C, or of ascorbic acid administered as a medicament.[1] The data were obtained by deproteinizing plasma by means of tungstic acid and titrating immediately the reduced ascorbic acid present with 2:6 dichlorobenzenoneindophenol.

In working with infants, and when making repeated observations on the same individual, it is desirable to have a method which requires a minimum of blood. We have, therefore, developed a micromethod requiring but 0.3 ml. blood, which may readily be secured from the finger of an adult, or the heel of an infant. In the guinea pig, blood is obtained by cardiac puncture, or sometimes by incision of the marginal vein of the ear. In the latter case, a special blood pipette is required.

Collection of Blood. Collect approximately 0.3 ml. blood from a lancet wound, into a small phial (Fig. 1, B) containing sufficient powdered potassium oxalate to prevent coagulation. Stir immediately with a toothpick or small glass rod. The phial is made by sealing the end of a piece of glass tubing of approximately 10 mm. outside diameter, and, while hot, pressing it against a piece of iron to form a flat base. The tubing is cut off at about 15 mm. from the closed end. The cut end is then ground flat on a fine stone wheel and the outer wall ground to a taper for a distance of 5 mm. from the top. The grinding produces a thin edge which greatly facilitates the collection of blood from the heel of an infant. It is unnecessary to grind the side wall if the phial is used for finger blood only (which is permitted to drop directly into the phial as the finger is milked), or if blood is transferred from a syringe. The phial is stoppered (Stopper A), then placed in a suitable holder (a cork with a recess cut in it) and lowered into the metal tube of a centrifuge. It is centrifuged for a few minutes in the usual manner.

The pipette C (80 mm. x 5 mm. O.D.) is closed to a fine opening

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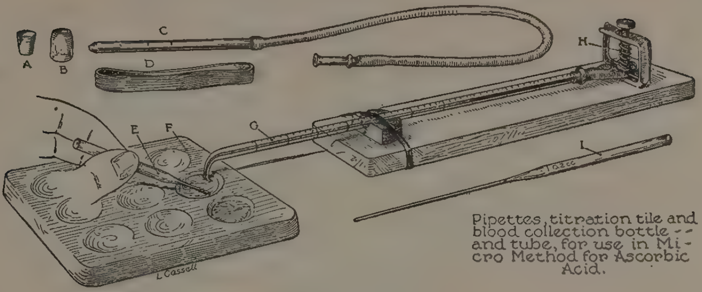
[1] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.



Pipettes, titration tile and blood collection bottle -- and tube, for use in Micro Method for Ascorbic Acid.

FIG. 1.

at one end and ground to a taper. It may be used to collect blood from the marginal vein of the ear in some guinea pigs having large, flat, vascular ears. A few crystals of potassium oxalate are placed in the tube, the rubber mouthpiece attached, and blood collected as when making a count. The graduations are at 0.3 ml. and 0.4 ml. capacity. When sufficient blood is obtained, detach the rubber tubing, and slip the wide rubber band D (approximately 75 mm. x 10 mm.) over both ends. Lower this assembly into the metal cup of the centrifuge, and centrifuge in the usual manner.

Deproteinization. Pipette 0.1 ml. of plasma (by means of a 0.1 ml. capillary pipette similar to I) into a 15 ml. conical centrifuge tube. With the same pipette, add 0.1 ml. distilled water, thereby rinsing the pipette. Add 0.2 ml. of a freshly prepared 5% metaphosphoric acid solution (pipette I) and mix thoroughly by tapping the centrifuge tube against the hand. Centrifuge for a few minutes. The 0.1 ml. and 0.2 ml. capillary pipettes are drawn from tubing of suitable size, and accurately calibrated.

Titration: The reduced ascorbic acid in the deproteinized plasma is titrated with a standardized solution of sodium 2:6 dichlorobenzenoneindophenol which is prepared as previously described.[1] A special pipette (G) is required (if a Rehberg microburette[2] is available, it may be used instead of the pipette described here) which is made from a thick-walled capillary tube of 0.8 mm. to 1 mm. bore. The capacity of the graduated portion is exactly 0.1 ml., and is contained in a length of about 200 mm. Each scale division is 0.002 ml., *i. e.,* the 0.1 ml. is divided into 50 parts, and calibrated at 5 points by means of mercury. The pipette is drawn out and curved downward at the end, and the tip ground to a cone by means of a grinding wheel. A thin film of vaseline is applied to the ground tip before use.

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[2] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Vol. II, p. 15. Williams and Wilkins, Baltimore, 1932.

The pipette is read to the nearest 0.001 ml. To operate the pipette, a rubber sac from a medicine dropper is filled two-thirds full of clean mercury and cemented or tied to the end of the glass tubing. This is compressed by means of a small Hoffman screw pinch cock which has a few coils of spring wire (H) wound loosely around the screw. The wire is attached by solder to the top bar of the clamp and to the movable bottom bar. The spring takes up lost motion in the screw and permits an even advance of the mercury in the capillary without back flow. The pipette is mounted on a lead base, the Hoffman clamp being attached by solder. A suitably grooved cork and rubber band support the protruding end of the pipette, as shown in the drawing. To fill the pipette, a few ml. of standardized dye solution are placed in a test tube. The test tube is inclined and the pipette inserted (without tilting) until the curved tip dips into the dye. The screw is now turned, compressing the rubber sac, until the mercury fills the capillary and a minute drop falls into the dye. The screw is then turned in the reverse direction until the pipette is filled with the dye to the desired amount.

The titration is carried out by pipetting 0.2 ml. of deproteinized plasma into one of the depressions of a white porcelain tile (F). Dip the curved end of the pipette into the fluid in the tile. *While stirring* with a fine pointed glass rod (E), slowly turn the screw of the Hoffman clamp until the first *faint* pink color is obtained. This is usually a permanent end point. From the reading of the pipette (minus the blank) and the standardization figure of the dye, the reduced ascorbic acid content of the plasma is calculated in milligrams per 100 ml. The blank is run by pipetting 0.1 ml. of 5% metaphosphoric acid solution and 0.1 ml. distilled water into a depression of the tile and titrating to the first permanent pink. The end point may easily be judged if 0.2 ml. of distilled water is pipetted into an adjacent depression in the tile plate. After titration, the pipette should be rinsed and left filled with distilled water.

The constancy of results obtained by this method is shown in

TABLE I.

Ascorbic Acid Solution Taken ml.	Blank ml. dye	Dye ml.	Ascorbic Acid Recovered mg.
.2	.005	.043	.00106
”	”	.042	.00103
”	”	.043	.00106
”	”	.043	.00106
”	”	.044	.00109
”	”	.043	.00106
Theoretical value			.00110

Table I, where a series of titrations were made on an ascorbic acid solution of known value. The ascorbic acid was dissolved in 2% metaphosphoric acid.

The use of metaphosphoric acid as a deproteinizing agent possesses many advantages over tungstic acid. Since we started to employ it (about 6 months ago), a paper appeared by Fujita and Iwatake[3] containing data on the relative stability of ascorbic acid in 2% metaphosphoric acid, 5% and 10% trichloracetic acid solutions, and water. They show no loss to occur in metaphosphoric acid for a period of 5 hours, whereas marked loss occurred in all other solutions. We have confirmed their results, and find no loss to occur in plasma filtrates standing over night when deproteinized with metaphosphoric acid. We, therefore, have abandoned tungstic acid as a deproteinizing agent, and when employing the macro-method,[1] proceed as follows:

Pipette 2 ml. of oxalated plasma into a round bottom 15 ml. centrifuge tube. Add 4 ml. of distilled water, followed by 4 ml. of 5% metaphosphoric acid solution. Thoroughly mix the contents of the tube, and centrifuge. Pipette 2 ml. portions of the deproteinized plasma into round bottom centrifuge tubes and titrate as described,[1] using a 5 ml. micro-burette. The titration may be made at any convenient time, but the plasma should be deproteinized as soon as possible.

In Table II, data are given showing the stability of ascorbic acid in plasma deproteinized with metaphosphoric acid.

TABLE II.
Ml. Dye Reduced by 0.2 ml. Metaphosphoric Acid Deproteinized Plasma.

Titrated Immediately* ml. dye	Titrated after 24 hours† ml. dye
.010	.010
.012	.012
.010	.010
.010	.010
.014	.012
.010	.010
.009	.010

*Samples taken from same person, at 30 minute intervals.
†After standing in refrigerator at 4° C. for 24 hours.

In Table III, we present data obtained on plasma, when 2 ml. samples were deproteinized by metaphosphoric acid, and the reduced ascorbic acid estimated by titration of a 2 ml. portion using a 5 cc. micro-burette, and a 0.2 ml. portion titrated by means of the micro-pipette described in this paper.

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[3] Fujita, A., and Iwatake, D., *Biochem. Z.*, 1935, **277**, 293.

TABLE III.

Plasma Sample No.	Ascorbic Acid Mg. % 2 ml. Portion	Ascorbic Acid Mg. % 0.2 ml. Portion
1	1.75	1.82
2	0.70	0.84
3	0.98	1.12
4	0.54	0.53
5	1.35	1.15
6	0.86	0.76
7	0.63	0.77

Although the values are not identical, they are in as close agreement as can be expected considering the absolute amounts of ascorbic acid contained in the samples being titrated.

We wish to acknowledge our indebtedness to Merck and Company, Inc., for a grant supporting this investigation, and to Miss Elizabeth Smith for technical assistance.

8536 P

Pressor Reactions and Gastric Ulcer.

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The local effect of single and repeated spasm of the small blood vessels apparently ultimately results in alterations in the tissues that can be demonstrated objectively. This has been shown by us in production of experimental bacterial endocarditis and also observed in studies of bacterial localization in the central nervous system. Vascular spasms were induced by single and repeated injections of pitressin (betahypophamine).

Our experiments were carried out on dogs and rabbits. The dose most useful for our purpose was found to be 20 pressor units per 5 kg. of weight of the dog and 10 pressor units per 2 kg. of weight of the rabbit. The pitressin was administered intravenously from one single dose to 50 doses in dogs and from 5 to 19 doses in rabbits. The repeated injections were given twice a week. Five dogs and 2 rabbits died, the rest were killed at certain time intervals.

The gastrointestinal tract was examined and the following data obtained:

In 12 of the 35 dogs there were macroscopic pathological changes in the stomach (multiple hemorrhages, erosions and small ulcers), one of these 12 dogs revealed a typical large stomach ulcer; in one dog there were lesions in the duodenum with multiple hemorrhages and erosions.

Of 18 rabbits, 8 revealed similar findings. Among these perforated ulcer of the stomach was observed in 2 cases.

Macroscopically and microscopically, the pathological lesions produced in the gastrointestinal tract of the animals resemble those seen in human cases.

These findings coincide with the results obtained by Dodds, *et al.*,[1,2] in their observations on the effects of the acetone picric acid extract of the posterior lobe of the pituitary body in monkey, cat, rabbit, guinea pig, rat and mouse.

8537 C

Hematologic Studies on Gastrectomized Monkeys

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During the course of our studies of the effects of gastrectomy on various species of animals, 8 of a group of 10 *Macacus rhesus* monkeys were gastrectomized, 2 being retained as controls. The age at which gastrectomy was performed could not be ascertained.

After recovery from the operation the same varied diet which included milk, bananas, oranges, apples, boiled potatoes, carrots, peanuts, etc. was fed the gastrectomized and control monkeys. Blood studies consisting of red blood count, hemoglobin (Newcomer), hematocrit and Price-Jones counts were made at frequent intervals. One X-ray film of the bones was made of each monkey.

Our studies showed that after an initial weight loss that lasted for approximately 80 days the gastrectomized monkeys slowly gained in weight to become relatively constant about 240 days after the operation. Five of the 8 gastrectomized monkeys are still alive 606, 396, 356, 347, and 228 days after operation. Three of the 5 have not regained their preoperative weight level, but are apparently in good health. The gastrectomized monkeys had an

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[1] Dodds, E. C., Noble, R. L., and Smith, E. R., *The Lancet*, 1934, **2**, 918.

[2] Dodds, E. C., Hills, S. M., Noble, R. L., and Williams, P. C., *The Lancet*, 1935, **1**, 1099.

oligocythemic, hypochromic, normocytic anemia for about 180 days after operation. Two monkeys, more anemic than the others, were each given a 0.1 gm. ampoule of ferric ammonium citrate subcutaneously every other day for one month. Their blood pictures immediately improved markedly and have remained relatively constant. At this time the blood pictures of the gastrectomized and control monkeys are comparable.

TABLE I.

	No. of animals	RBC	Hb	Hemato-crit	Price Jones
Gastrectomized monkeys	5	5,90	12.63	46.0	6.99
Control monkeys	2	6,32	12.94	47.5	6.80

The blood picture for 11 normal monkeys was (Table II):

TABLE II.

Determination	No. of determinations	Mean	Range
Red blood count	24	6,20	(4,72-7,66)
Hemoglobin (Newcomer)	24	12.86	(9.00-17.27)
Hematocrit	24	47.64	(39.0-55.75)
Price-Jones count	23	7.04	(6.79-7.75)

No differences between the bones of the gastrectomized and control monkeys were detected in the X-ray films.

Thus, gastrectomized *Macacus rhesus* monkeys, like the dogs, pigs and rats, do not develop pernicious anemia after gastrectomy, at least, within from 1 to 2 years. However, an iron deficiency anemia, which also appears in dogs, pigs, and rats, may result.

8538 P

Fundusectomy Prevents Post-Operative Jejunal Ulcer

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The irritating action of gastric juice, the nutritional state of the animal and the greater susceptibility of jejunal mucosa to irritation by acid (as compared to duodenal mucosa) are the principal factors concerned in the development of jejunal ulcers in dogs prepared by the Exalto-Mann-Williamson technique. To study the relative rôles played by gastric juice and nutrition the following experiments were performed:

Control Series with "Stock Diet." A group of 42 control dogs were operated by the Mann-Williamson technique and fed a diet consisting of ground meat (parboiled), milk, bread, and cod liver oil. All dogs died with a jejunal ulcer within a period of 17 weeks.

Fundusectomy with "Stock Diet." Eleven dogs were fundusectomized (three-fourths of fundus removed) and then three weeks later the Mann-Williamson operation was performed. The maximum survival time after the second operation was only 15 weeks, but the incidence of ulcer was only 27 per cent. The dogs lost weight rapidly, having been deprived of the greater part of both gastric and pancreatic digestion. It thus became obviously necessary to feed a diet more easily digested and absorbed.

A Series in which Nutrition was Improved: (Special diet). Twenty dogs were similarly operated and fed a more easily assimilated diet consisting of finely ground cooked meat, wheat and barley flour, dried milk, tomato juice, bonemeal, cod liver oil, fresh whole milk, raw ground pancreas (200 gm. daily), fresh ground liver (200 gm. daily), corn syrup (100 gm. daily) and banana flour. This diet was fed three or four times a day, some dogs eating as much as from 4 to 6 lbs. daily. Ten of the dogs lost weight from the start; but only three died with ulcer within 17 weeks, the others dying from other causes than ulcer. Nine of the remaining ten survived from 20 to 58 weeks, but died with ulcer. The other dog was anesthetized at 82 weeks and no ulcer was found. Only two of these dogs lost weight appreciably, most of which resulted after the ulcer started to bleed.

Thus, it is evident that the improvement of nutrition definitely delayed the onset of ulcer and delayed perforation or lethal hemorrhage.

Fundusectomy plus the Special Diet: Thirteen dogs were fundusectomized and placed on the special diet. Three or four weeks later the M. W. operation was performed and the special diet continued. All but one survived from 17 weeks to 2 years; none developed ulcer. Only three lost weight but did not develop ulcer. Seven survived longer than 1 year and 3 months, and six survived more than two years. The gastric acidity returned to normal control values approximately (test meal) in from 6 to 8 months, although the acidity values in two of the six dogs at 2 years were low normals. When the *volume production of acid* in response to histamine in five of the six dogs surviving two years was studied, it was found to be similar to that of normal dogs, but about one-half of that of the Mann-Williamson dogs. The emptying of the stomach is approximately normal.

Conclusion: Fundusectomy plus a special, easily assimilated, nutritious diet prevents jejunal ulcer (occurrence otherwise from 95-100 per cent) from occurring in dogs having a gastro-jejunostomy and drainage of bile and pancreatic juice into the last 15 cm. of the ileum. The feeding of the special diet alone to dogs so operated only delays the onset of the ulcer, perforation and lethal hemorrhage. This shows that acid is a more important factor in the cause of this type of ulcer than nutrition.

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New York Academy of Medicine, March 18, 1936.

8539 P

General and Cerebral Anaphylaxis in the Monkey.

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Lenore M. Kopeloff.

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Monkeys have rarely been used in anaphylactic experimentation because they were found to be refractory. Zinsser[1] concluded that "anaphylaxis is very difficult to obtain in the lower monkeys, probably cannot be obtained by a single preparatory injection, but that occasionally definite mild anaphylactic reactions can be obtained in these animals." In continuing our studies on local cerebral anaphylaxis[2] we proceeded in the *Macacus rhesus* as in the dog, namely, with intravenous injections following repeated or continuous antigen contact in the brain. Four out of 7 monkeys (Nos. 3, 5, 8, and 9) responded with slight contralateral loss of tone in the hind leg following intravenous injections of the appropriate antigen.

One animal (14) however, which had been previously sensitized with egg-white intramuscularly and intravenously and subsequently injected directly into the left motor area of the brain with the same antigen responded 2 days later with striking convulsive twitchings of the right arm and right side of the face. The right motor area of the brain had received horse serum as a control but it failed to cause any reaction.

Another series of monkeys were given repeated intravenous injections of antigen. In these fatal anaphylaxis occurred.

Table 1 shows that 11 of the 12 monkeys died of acute anaphylactic shock. (The exception was a monkey which was given a concomitant intravenous injection of 1 cc. adrenalin—marked gen-

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[1] Zinsser, H., Proc. Soc. Exp. Biol. and Med., 1920, **18**, 57.

[2] Davidoff, L. M., and Kopeloff, N., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 71; 1934, **31**, 980; *J. Lab. Clin. Med.*, 1935, **20**, 1238.

TABLE I.
Results of Injections of Egg-White in Monkeys.

No.	Preliminary Treatment	Subsequent No. Injections E.W. i.c.	No. Injections E.W. i.v.	Final Reaction
3	Agar c E.W. i.c.	0	4	Collapse*
4	,, ,, ,, ,,	4	1	Death
5	,, ,, ,, ,,	5	2	,,
6	,, ,, ,, ,,	3	2	,,
7	E.W. i.c.	2	3	,,
8	,, ,,	2	2	,,
9	,, ,,	2	2	,,
11	,, ,,	0	2	,,
16	E.W. i.v.	0	2	,,
24	,, ,,	0	3	,,
13	E.W. i.m.	0	2	,,
15	E.W. i.v.	1	1	,,

*1 cc. adrenalin injected concomitantly.
i.c.—intracerebral.
i.v.—intravenous.
i.m.—intramuscular.

eral weakness resulting, nevertheless). The order of injections, elapsed time, etc. have been omitted for the sake of brevity but will be included in a more extended report.

From the data summarized in Table 1 it appears that monkeys may be rendered anaphylactic by the administration of an antigen (egg-white) in at least 2 doses given at suitable intervals, so that a final shocking dose produces death regularly. In 3 others death followed a *single* preparatory dose.

In 4 monkeys (Nos. 4, 5, 6, and 15) previously given at least 2 injections of antigen (egg-white) a series of intracerebral injections of the same antigen into the motor areas at 2-day intervals produced marked local swelling at the site of injection and in 2 instances, death. At necropsy, definite aseptic necrosis of the brain was found in the area of injection.

8540 P

Toe Lesions Following Tobacco Injections in Rats.

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(Introduced by Louis Gross.)
From the Laboratories of The Mount Sinai Hospital, New York City.

During the past 5 years we have attempted to produce gangrenous lesions of the extremities in rabbits and cats by the injections of tobacco or nicotine. We hoped in this way to study the possible rôle played by tobacco in the etiology of thrombo-angiitis obliterans. The results have been consistently disappointing. Stimulated by the work or Rothlin[1] and McGrath[2] who produced gangrenous lesions in the rat's tail by the use of ergotamine, we decided to employ albino rats for our experiments with tobacco. This preliminary report deals with the findings in a series of 48 male and 12 female rats subjected to injections of tobacco extract. In addition, a group of animals was subjected to inhalation of tobacco smoke and another group was given injections of the pure alkaloid nicotine tartrate.

A Ringer's solution extract was prepared from a commercially denicotinized tobacco from which about 60% of the nicotine had been removed. The injections were given intraperitoneally once every day. The amount first injected was equivalent to one sublethal mouse unit. This was gradually increased so that by the end of the fourth week approximately 3 sublethal mouse units were administered daily.

Of 48 male rats treated with injections of denicotinized tobacco, 33 developed gangrene of the toes within 5 to 12 weeks. None of the 12 female rats treated for 5 to 18 weeks developed this lesion. In 10 control male animals, living under identical conditions, no gangrene developed.

The characteristic lesion begins with a blanching of the toe which is followed by intense redness and swelling. Within 24 to 48 hours of the onset of the blanching the terminal portion of the toe becomes gangrenous and sloughs away leaving an open ulcer. This heals with loss of tissue. Most of the animals showed involvement of one or 2 toes. In several of them all the toes of one or two feet were successively involved.

Preliminary microscopic studies showed an inflammatory process involving the vessels. Detailed studies are being carried on.

Of 6 rats subjected daily to inhalation of tobacco smoke, over a period of 5 months, one male animal developed lesions exactly like those produced by injections of the extract.

One male animal of a group of 10 treated with daily intraperitoneal injections of alkaloid nicotine tartrate also developed a characteristic lesion in one foot.

The fact that a gangrenous process could be produced only in the male is interesting in connection with the almost exclusive occurrence of thrombo-angiitis obliterans in men.

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[1] Rothlin, E., *J. Pharm. and Exp. Therap.*, 1929, **36**, 657.

[2] McGrath, Edward J. G., *Arch. Int. Med.*, 1935, **55**, 942.

8541 P

X-Ray Absorption Coefficients of Coronal and Root Dentin.*

Franklin Hollander and Edmund Vesely

From the Laboratory of Physical Chemistry, Department of Oral Histology, Columbia University Dental School, New York.

By means of the precision micro-method recently reported[1], linear X-ray absorption coefficients were measured in various parts of the dentin of 4 non-carious human teeth (3 third molars, 1 premolar) from different individuals of ages from 17 to 68 years. The ground sections were approximately 300 micra thick and the disc-shaped areas of observation were 1 mm. in diameter. A photomicrographic record of each section made it possible to correlate values of the coefficient with the structural characteristics of each disc. The number of independent measurements per tooth varied from 10 to 20, depending on the size of specimen; each measurement was done in duplicate.

The coefficients for each specimen showed considerable variation (as great as 20%) among themselves. Their mean values were 95.4 ± 0.9 units,† 96.3 ± 0.7, 94.1 ± 1.2 and 95.2 ± 0.6 respectively; even the greatest difference between these means is so small that its significance is statistically uncertain. It is worthy of note that the mean values for the 2 halves of any one section, taken with respect to its longitudinal axis, are unquestionably identical; the differences between such means were 0.2, 0.8, 0.8, and 0.1 units, or less than 1% of the means themselves. The maximum values for the several specimens (101.5, 100.7, 99.6, and 99.6) were so close together that any difference between them is likewise of questionable significance. Thus, for the several specimens studied, both the mean and the maximum values differ by no more than 2%, an amount so small as to make the values practically identical. On the other hand, their minimum values varied significantly (89.3, 86.8, 79.3, and 81.7), due in part to structural variations. As yet it is impossible to establish any quantitative correlation with type of structure, possibly because the number of sections is too few to permit of generalization in this regard.

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*Aided by a grant from the Commonwealth Fund of New York.

[1] Hollander, F., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 388.

† These units are expressed as micra of aluminum equivalent to 100 micra of tooth substance, in respect to absorption capacity. The precision measure is the probable error of the mean.

The distribution of the values of the coefficient in any one specimen was not random, but tends to decrease as the position of measurement passes from the crown toward the apex of the root. In order to establish this difference between coronal and root dentin conclusively, the values in these 2 regions were grouped and averaged for each specimen. The differences between the corresponding means for crown and root were 7.4 ± 1.0, 6.4 ± 1.3, 4.2 ± 1.0, and 2.2 ± 1.2 respectively. Consistent with the occurrence of this gradient, the coronal values were scattered far less than were the root values—using the probable error as a criterion of scatter.

It appears, therefore, that the coefficient approaches an upper limit in the coronal dentin of non-carious teeth—which is in fairly close agreement for all 4 specimens—and that the deviation from this maximum value tends to increase with the distance between the position of measurement and the dentino-enamel junction. Dentin formation is known to start at this junction and to proceed toward the root apices. Thus, the gradient in the X-ray absorption coefficient of the dentin appears to parallel the progress of its formation. Whether the gradient results from the pre- or post-eruptive suspension of the calcification process cannot yet be inferred from these observations.

8542 C

Volume Concentration of Rat Muscle Measured by the Phosphate Partition Method.

CHARLES A. WINTER AND ERIC PONDER.

From the Department of Physiology, University of Buffalo, and The Biological Laboratory, Cold Spring Harbor.

A simple method, based on the assumed impermeability of muscle cells to phosphate, has recently been described for finding the volume concentration (relative volumes of interspaces and cells) in frog muscle.[1] This investigation was undertaken with the idea that the method might be applicable to rat muscle.

The method used was essentially that of M. G. Eggleton. Adult rats were decapitated, and paired leg muscles (extensor digitorum longus or tibialis anticus) were quickly dissected out and weighed.

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[1] Eggleton, M. G., *J. Physiol.*, 1933, **79**, 31.

One muscle of a pair was placed in mammalian Ringer's solution of a given phosphate content, and the other in a solution of a different phosphate content. The phosphate (4 parts Na_2HPO_4 to 1 part KH_2PO_4) was added in M/15 concentration to insure isotonicity. The muscles were left in the solutions for 1, 2, 3, or 9 hours, and at the end of the period they were again weighed and the P of the solution was determined by the method of Martland and Robison.[2] From the weights of the muscles and from the initial and final phosphate concentrations in the Ringer's solution, one can calculate *c,* the initial concentration of phosphate in the interspaces, and *a,* that fraction of the muscle water contained in the interspaces.[3] The volume concentration is (1-*a*), and Eggleton found a mean value of about 23% for frog muscle.

In many cases results similar to Eggleton's were obtained (Table 1.) In these experiments, *a* has the mean value of 0.35 and varies between 0.20 and 0.53. In other experiments (Table II), however, unreasonable or even impossible results were found for *a.* (The two tables do not represent different series of experiments;

TABLE I.

Original P in Ringer, mg./100 cc.		Duration of experiment, hrs.	Final P in Ringer, mg./100 cc.		*a*	*c*
low	high		low	high		
10	20	1	11.3	21.0	.43	0.54
10	20	1	11.1	21.0	.31	0.83
30	40	2	30.0	39.7	.41	0.30
20	40	1	22.0	41.3	.20	0.85
20	40	1	22.3	40.8	.53	0.52
10	30	1	11.0	30.5	.51	0.37
20	40	1	20.6	40.3	.26	0.52
10	40	1	11.1	40.3	.36	1.49
30	60	9	32.9	62.2	.25	1.69
30	60	9	31.9	61.2	.30	1.24

TABLE II.

Original P in Ringer, mg./100 cc.		Duration of experiment, hrs.	Final P in Ringer, mg./100 cc.		*a*	*c*
low	high		low	high		
30	40	2	29.7	39.7	—0.03	1.90
30	40	2	29.9	39.7	—0.14	0.39
10	60	3	11.6	61.8	—0.02	18.80
10	30	1	11.0	31.1	—0.05	—1.93
40	80	1	40.3	80.8	—0.22	—0.16
40	80	1	41.1	80.0	0.08	1.67
40	80	1	40.8	80.8	—0.01	—7.33
30	60	9	30.6	60.8	—0.17	0.60

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[2] Martland, M., and Robison, R., *Biochem. J.,* 1926, **20,** 847.

[3] The equations are given in Eggleton's paper. Through what seems to be a misprint, *c* is there expressed in mg. per 100 g., instead of in mg. per g.

we have merely separated the results which are comparable with Eggleton's from those which are not). Indeed, an inspection of the figures in Table 1 raises the question as to whether even these are reliable, for the scatter is very great. A similar scatter in the value of *a* appears in Eggleton's experiments on frog muscle, and close inspection shows that large variations in *a* and *c* are very apt to be inherent in the method and can scarcely be avoided. This is because a small error in the phosphate determination makes such a great error in *a* and *c*; for example, in one experiment we found *a* to be 0.25 and *c* to be 1.69, but if a 1% error is allowed in the phosphate determination, the true value of *a* might work out at any figure between —0.12 and 0.63, while that of *c* might be anything from 2.19 to 0.93. To get reliable values for *a* and *c*, it is necessary to have the diffusion of a large amount of phosphate determined with great accuracy, and in the case of rat muscle at least, this large amount does not diffuse; considerations of this kind can easily explain the variations in *a* both in our experiments and in Eggleton's.

Unlike the behaviour of frog muscle as reported upon by Eggleton, there was always some gain in weight in isotonic Ringer's solution by the muscles in our experiments, irrespective of the fact that they were immersed in the phosphate solutions at about 2°C. This probably indicates either a change in the osmotic pressure within the muscle as a whole, or post-mortem changes in some of the cells only, and it is impossible to be sure that the latter do not occur. Some of our values for *a*, and some of Eggleton's too, are so high that they are best accounted for on the grounds that some of the cells are permeable to phosphate, perhaps as a result of such post-mortem changes, and the fact that most of our rat muscles gave a feeble response to electrical stimulation even after only one hour immersion points in the same direction.

Conclusion. The phosphate partition method used by Eggleton for finding the volume concentration in frog muscle is not satisfactory when applied to rat muscle (extensor digitorum longus and tibialis anticus). The variability obtained appears to be due partly to the extreme sensitiveness of the results to small errors in phosphate determination, and partly to the fact that complete impermeability of the muscle cells, under the conditions of the experiments, is extremely doubtful.

8543 C

Effect of Quinine Hydrochloride on Resistance of Rabbit Red Cells.*

ERIC PONDER AND JULIUS C. ABELS.
From The Biological Laboratory, Cold Spring Harbor.

Clinical observations on blackwater fever have suggested either that red cells of an individual treated with large doses of quinine salts are unusually susceptible to intra-vascular lysis, or that quinine salts act as lysins for red cells already weakened by intra-vascular lysins which appear in the disease. The purpose of this paper is to show that red cells of rabbits receiving quinine hydrochloride in proper amount are less resistant to lysins such as saponin and sodium taurocholate used *in vitro*. The experiments fall into 2 groups: (a) *in vitro* experiments, which show that quinine hydrochloride is a simple hemolysin, and (b) *in vivo* experiments, which show that the resistance of the animal's red cells is lessened by the administration of the quinine salt.

(a) *In vitro experiments.* The time-dilution curve for quinine hydrochloride and rabbit red cells can be found in the usual way,[1] and, at 25°C., is:—

Dilution, 1 in	25	30	40	50	60	100
t, minutes	3.0	4.0	9.5	17	25	85

This is a typical time-dilution curve for a simple hemolysin, and the endpoints are sharp and regular. As might be expected, the addition of quinine hydrochloride to systems containing saponin or sodium taurocholate produces a marked acceleration of hemolysis, and this can be measured by the usual methods.[1] If the cells are left in contact with 1 in 100 quinine hydrochloride for 10 minutes (after which time there is no lysis), and then washed and re-suspended, the resulting suspension has a smaller resistance to saponin and taurocholate than has an untreated suspension, and the R-values which measure the acceleration are about 0.70 to 0.75. The simplest explanation of what happens is that the quinine salt combines with some component of the cell membrane,[2] transforming it sufficiently to bring about hemolysis if the concentration of the

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*This work was carried out with the aid of a grant from the National Research Council to one of us. (E. P.).

[1] Ponder, E., "The mammalian erythrocyte and the properties of hemolytic systems," Protoplasma Monographien, No. 6, Borntraeger, Berlin, 1934.

[2] Gibbs, O. S., *J. Pharm. and Exp. Ther.*, 1928, **33**, 210.

quinine is great, and transforming it partially, but sufficiently to decrease its resistance to a subsequently added lysin, if the concentration of quinine is less. The phenomena seem to be like those observed in the case of the "additive" effect of 2 lysins.[3] In all cases the pH of the quinine solution was between 6.0 and 7.0, and so the effects cannot be attributed to H ion.

(*b*) *In vivo experiments.* In these experiments quinine hydrochloride was given to rabbits in varying dosage and over various periods, and the resistance of the animal's red cells to saponin and to taurocholate was measured at intervals. The quinine salt was given by daily subcutaneous injection in doses of from 20 mg. to 250 mg. per kilo, and samples of blood were taken by heart puncture, 2 cc. being withdrawn daily every 5 days in routine experiments, but more often when the dose was expected to be lethal. So that all suspensions to be used for determining red cell resistance should be comparable, red cell counts were made on each sample of blood, and the suspensions were prepared so as to contain 1.25×10^8 cells/cc. Reticulocyte counts were carried out in wet mounts.

The cell resistances were determined by a simplified method.† A dilution of saponin in 1% NaCl (1 in 20,000), and one of taurocholate in 1% NaCl (1 in 1,200, pH = 7.3) were selected as the lysins with respect to which resistance was to be measured, for these dilutions produce complete lysis of an average rabbit red cell suspension containing 1.25×10^8 cells/cc. in about 10 min. at 25°C. The hemolytic systems always contained 0.8 cc. of the lysin in the dilution just stated, 0.8 cc. of isotonic NaCl, and 0.4 cc. of the cell suspension. As a check on the stability of the lysins, a determination of the resistance of the cells of our own blood (J.C.A.) was made on each occasion. The resistance of the cells of the various samples of rabbit blood is expressed as the time required for complete lysis in the systems described.

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[3] Ponder, E., *Biochem. J.*, 1934, **28**, 384.

†The proper method of comparing resistances is to compare in their entirety 2 time-dilution curves, one for the lysin acting on the cells alone, and the other for the lysin acting on the cells in the presence of the accelerator or inhibitor. This method is very laborious, and is quite unnecessary in connection with this investigation. We have accordingly compared the times for complete lysis, under more or less standard conditions, for the cells of the animal at different stages of the quinine administration. It ought to be pointed out, however, that such a comparison gives an exaggerated idea of the change in cell resistance; in Table II, for instance, we have reductions in times for lysis from 10 min. to 3 min., but this corresponds to a resistance change of only some 30%, and not to one of some 300%.

The results can be divided into 3 groups, depending on the amount of quinine hydrochloride administered.

Group 1. Dose 20-50 mg./kilo/day. With small doses (20 mg./kilo/day), there is no significant effect either on the red cell count or on the red cell resistance over a period of at least 15 days. If at the end of this time, however, the dose is increased to 50 mg./kilo/day, there is a sharp rise in the number of reticulocytes, a slight (10%) fall in the red cell count, and a decrease in the red cell resistance (decrease in the time for lysis from about 12 min. to about 8 min.). These changes are transient, and with a continuation of the 50 mg./kilo/day dose the count and number of reticulocytes tend to return to the original values. The cell resistance, however, remains low until the quinine is discontinued, after which the original resistance is attained in from 15-20 days.

Group 2. Dose 75-125 mg./kilo/day. Within 5 days there is a sharp rise in the reticulocyte count, a 10% fall in the red cell count, and a decrease in the time for lysis from one of about 12 min. to one of about 8 min. With continued administration, the red cell count falls another 20% within a further 10-day period, and the resistance decreases to a time for lysis of about 4 min. The reticulocyte count falls to its usual value, the sharp rise which occurs

TABLE I.

Day	Dose, mg./kilo	Red cell count, $\times 10^{-3}$	Reticulocytes, %	Resistance to saponin, min.	Resistance to taurocholate, min.
0	0	5,756	0.3	12	10
5	75	5,240	1.2	8	7
10	75	4,444	0.6	5	4
15	75	4,020	0.2	4	4
20	0	4,982	0.4	6	7
25	0	5,578	0.3	10	10
30	0	5,802	0.3	12	11

TABLE II.

Animal	Max. fall in r.b.c.'s, %	Change in resistance to saponin, min.		Change in resistance to taurocholate, min.	
		From	To	From	To
1	26	9.5	4.5	—	—
2	31	10.0	2.5	10.0	4.2
3	22	10.0	3.0	10.0	4.0
4	30	10.3	3.2	11.5	4.5
5	31	10.5	3.2	11.0	4.0
6	30	12.0	4.0	10.0	4.1
7	23	10.0	3.0	10.5	3.5
8	32	11.5	2.2	10.5	2.0
9	31	12.5	2.0	10.0	2.5
10	26	10.3	3.1	10.8	2.9

initially apparently representing a transitory marrow activity which later ceases. As an illustration, the results of a typical experiment are given in Table 1, and a summary of the principal results for 10 rabbits in Table II.

Group 3. Dose greater than 150 mg./kilo/day. Doses of about 170 mg./kilo/day produce diarrhoea, and are usually fatal within a few days, while doses as great as 250 mg./kilo/day often produce death within 24 hours. The fall in the red cell count is not as great as it is in the case of animals of Group 2, principally because the animals do not live long enough; there is always marked decrease in red cell resistance, however, the time for lysis being reduced from about 12 min. to about half that time.

These results can be simply accounted for on the basis of quinine hydrochloride being a simple hemolysin with properties similar to those of simple hemolysins in general. Thus *in vitro* it is lytic in sufficient concentration, and in smaller concentration it is an accelerator of saponin and taurocholate hemolysis (the "additive" effect of 2 lysins). *In vivo,* successive administrations bring about a decreased resistance of the red cells to saponin and taurocholate used *in vitro,* and the fall in the red cell count which occurs with suitable doses of the drug is probably due to an intra-vascular lysis, brought about by intra-vascular lysins present in the blood stream (e.g., the bile salts and the unidentified lysins of urine), the action of which is accelerated by the quinine. It is unlikely that the quinine salt itself produces the intra-vascular lysis, for the greatest concentration in which it could exist, under conditions in which the effect occurs, is about 1 in 1000, and this concentration would not be lytic even for cells in saline.

8544 C

Bactericidal Power of Dried Painted Surfaces.* (I).

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The development of a paint which has bactericidal properties after drying has been reported recently by Wetchler, Lewis and Battline.[1] This paint has, as the binder, a 4% halogenated oil base. The present study deals with bactericidal tests on this type of paint after prolonged periods of drying in comparison with a number of ordinary commercial paints bought in the open market. The cultures used for testing have been *B. typhosus,* "Hopkins" (U.S. Food and Drug Administration strain), and *Staphylococcus aureus,* 4 strains, namely, No. 209 (U.S.F.D.A. strain), No. 3 (stock strain of the Bureau of Laboratories, N.Y.C. Dept of Health), and 2 freshly isolated from boils. Work on bactericidal paints has been reported by Portier and Kling,[2] Trouissant,[3] and Vallée.[4]

Two methods were used for determining bactericidal action as judged by the lack of growth on media. The method on which the majority of results given in this paper were based, consisted in the use of painted glass rods, 10 mm. in diameter, which were allowed to dry in the air for various periods of time. Baking at 100° C. and 125° C. was employed to simulate longer periods of air-drying; this method of aging is a common practice in paint technology. For the tests, the rods were immersed in test tubes containing 4 cc. of saline (0.85%) suspension of the culture (18 hour agar slant) and kept at room temperature for a given time. The rods were then removed and 0.2 cc. of the culture suspension placed on the surface of extract agar plates. Observation of the amount of growth was made after 18 and 42 hours' incubation at 37° C. Prolonged incubation for 4 days did not show any appreciable increase in the number of colonies. Dilution pour plates were made in a number of instances to eliminate the possibility of an inhibitory factor which would be removed by further dilution. The density of the bacterial suspension used in each test was determined by comparison with barium sulfate standards and by plate count made from serial dilution in salt solution.

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*This work was made possible through a grant from the Camel Lead, Color and Chemical Products Mfg. Corp., Brooklyn, N. Y.

[1] Wetchler, S., Lewis, A., and Battline, F., *Paint and Varnish Production Manager,* 1936, **14**, 12.

[2] Portier, P., and Kling, A., *Bull. Acad. de Med.,* 1931, **406**, 305.

[3] Trouissant, *Bull. Acad. de Med.,* 1933, **109**, 448.

[4] Vallée, *Chemie et Industrie,* 1934, Special No. 962-968.

In the second method 0.1 cc. of the saline culture suspension was placed on a square inch area of a painted tin disc, and separated into small drops with the aid of a loop. The discs were then placed in petri dishes containing a layer of filter paper moistened with 1 cc. of water and allowed to stand at room temperature for 6 hours; then 0.3 cc. of sterile salt solution was pipetted onto the area, rinsed up and down 3 times, and placed on the surface of extract agar plates. These plates were then incubated and observed for growth as in method 1.

Experiments with *B. typhosus.* Method 1. The results of the tests of 2 halogenated paints, A and C, and the 10 commercial paints, D through P, with *B. typhosus* (*B.t.*), carried out according to method 1, are summarized in the table. The 3 commercial paints, D, E, F, were used for comparison in the majority of tests; later, 7 additional paints were studied. Painted rods were tested after air-drying for periods from 2 days to 9 weeks, and after baking at 100° C. and 125° C. for periods up to 6 hours. The results of these experiments showed that the dried painted films of paints A and C exerted a marked bactericidal effect upon saline suspensions of *B.t.* in the test tube, as judged by the lack of growth on agar plates. The painted surfaces were still effective after air-drying for 9 weeks and after baking for 6 hours at 125° C., an approximate equivalent of 6 years of air-drying.† In all of the tests with paints A and C, the growth was either completely inhibited or the initial viable population of the culture was reduced from 15-80 million colonies per cc. to 250-1000 per cc.

Of the 10 commercial paints, 7 had bactericidal power 2 days after painting but by the tenth day only 4 were slightly germicidal. When tested 6-7 weeks after painting, paints D and O remained somewhat effective; and, after baking at 100°C. for 6 hours, O alone showed very slight bactericidal action.

Method 2. A limited number of experiments were performed with painted tin discs after air-drying for 2 days and 7-8 weeks, and baking at 100°C. for 6 hours, an approximate equivalent of

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†The rate of a chemical reaction is increased from 2 to 3 times for every 10 degree rise in temperature Centigrade. The average is used here to arrive at the approximate time-equivalent of baking and air-drying.

380 days of air-drying. The results were as follows: after 2 days of air-drying, paints A,C,D,F,G,H,K,L, and O were entirely bactericidal; paints E,J, and P showed no growth inhibition. When air-dried for 7-8 weeks, A,C,D,G, and O were strongly bactericidal while the rest showed no, or very slight, inhibition of growth. After 6 hours at 100°C. the halogenated paints A and C were completely bactericidal and all of the commercial paints were devoid of germicidal activity. These results with the painted discs, air-dried for 2 days, 7 weeks or baked at 100°C. for 6 hours coincided closely with those obtained with the rods, the only variation being the evidence of germicidal power by paints D and O when air-dried for 7 weeks.

Experiments with *Staphylococcus aureus.* Method 1. The greater resistance of *Staphylococcus aureus* (*S. a.*) to disinfectants, as shown in phenol coefficient tests, was also apparent in the preliminary tests of this organism with the halogenated paints. These tests showed that the time of exposure (6 hours) effective in the case of *B.t.* was insufficient for *S.a.*

Twenty-two experiments were carried out with *S.a.* No. 209 and No. 3 in which the time of exposure of the painted rod and culture suspension varied from 16-24 hours and the strength of the culture suspension from 15-115 million colonies per cc. The rods were tested after air-drying for periods up to 7 weeks and after baking at 100°C. for 45 minutes, 2 and 4 hours. The results of these tests did not show the consistent and marked bactericidal effect that had been found with *B.t.* The halogenated paints A and C were strongly germicidal in 16 and 15 experiments, respectively, out of 22. In all of these tests, paints A and C showed greater action upon the bacteria than the commercial paints E and F, but were only slightly better than paint D. Preliminary work on a slight modification of paints A and C has shown encouraging results with *S.a.* No. 209 after 6 hours' exposure. Further experiments on this paint are in progress.

Wetchler, Lewis, and Battline[1] reported strong and consistent bactericidal action upon a staphylococcus isolated from the throat. This strain was found to be somewhat atypical in growth characteristics and therefore it was not included in the experiments presented here.

Summary and Conclusions. Two halogenated paints and 10 commercial paints were tested for bactericidal action after drying in the air for periods up to 9 weeks and after baking at 100°C. and 125°C. for periods up to 6 hours, approximate equivalents of 380

TABLE I.
Bactericidal Tests of Painted Rods—*B. typhosus.*

Method of Aging		Air-Dried				Baked					
						100° C.			125° C		
Time of drying or baking		2 days	7-10 days	6-7 wks.	9 wks.	6 min.†	1,4-hr.	6 hr.	30 min.	1 hr.	6 hr.
Strength of bacteria in millions, per cc.*		50-140	80-150	80	68	115	80	80	25	25	15
Paint	Description										
A	Halogenated	0	0	0-tr	tr	0	0	0	tr	0	tr
C	,,	0	0	0-tr	0	0	0	0-tr	tr-+	0	0-tr
D	Gloss ivory	0-+1	++	+1	+1	+1	++	++	++	++	++
E	Enamel ivory	++	++	++	++	+1	++	++	++	++	++
F	Semi-gloss ivory	0-+1	+1-++	++	++	+1	++	++	+1	++	++
G	Gloss white	0	++	++	++			++			
H	China gloss white	tr	+1-++	++				++			
J	Gloss white	++	++	++				++			
K	Inside gloss white	tr	++	++				++			
L	Gloss white	0	++	++				++			
O	Inside gloss white	0	tr	tr-+1				+1			
P	Interior gloss white	++	++	++				++			
Control	No paint	++	++	++	++	++	++	++	++	++	++

*Colony count of culture suspension in which rods were immersed.

†Approximate equivalent time of air-drying:

At 100° C,
6 min. = 6⅓ days
15 ,, = 15½ ,,
45 ,, = 47 ,,
4 hr. = 253 ,,
6 ,, = 380 ,,

At 125° C,
30 min. = 6⅔ mo.
1 hr. = 400 days
4 ,, = 4½ yr.
6 ,, = 6½ ,,

Explanation of Symbols

0 = No growth on surface agar plates after 18 hr. incubation; none or less than 50 colonies after 42 hr. incubation.
tr (trace) = No growth or very slight growth after 18 hr. incubation; between 50 and 200 colonies after 48 hr. incubation.
+ = Slight growth after 18 hr. incubation; between 200 and 800 colonies after 42 hr.
+1 = Moderate growth after 18 hr. incubation.
++ = Heavy growth after 18 hr. incubation, equal to control.

The large majority of the tests tabulated were carried out on duplicate rods. Where 2 readings are given, the results of the 2 rods were different.

days and 6 years of air-drying respectively. The results of the experiments showed that glass rods, painted with the halogenated paints, after air-drying or baking for the time and temperature indicated, exerted a strong bactericidal effect upon saline suspensions of *B. typhosus,* when exposed for 6 hours at 20°C., as judged by the amount of growth on agar plates. Of the 10 commercial paints tested in the same manner, 7 were bactericidal 2 days after painting, but when tested 8 days later 9 had almost or completely lost bactericidal power; at the end of 6 weeks no appreciable germicidal property was manifested in any of them. Painted tin discs, air-dried for 2 days or 7 weeks, or baked at 100°C. for 6 hours gave results which coincided closely with those obtained with the rods.

Experiments with *Staphylococcus aureus* exposed to painted rods for 16-24 hours showed that the painted surfaces of the halogenated paints air-dried for periods up to 7 weeks, or baked at 100°C. for 45 minutes, 2 and 4 hours, were moderately but not consistently bactericidal. They were superior to the 3 commercial paints tested in comparison.

8545 C

Permeability of Germinal Vesicle of the Starfish Egg to Water.

LYLE V. BECK AND HERBERT SHAPIRO.
(Introduced by Robert Chambers.)

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While an enormous amount of research has been carried out on the permeability of the external cell membrane, very little consideration has been given to the permeability of the nuclear membrane. In fact, only one paper, that of Monne[1] on diffusion of microinjected dyes from cytoplasm to nucleus, has come to our attention.

A few preliminary experiments, carried out at the end of the past season at Woods Hole, indicate the possibility of making quantitative and extended studies on the permeability to water of the nuclear membrane in permanently immature eggs of the starfish, *Asterias forbesii.* The great advantage of this cell over others examined is that the nucleus is of a size sufficient to make possible fairly accurate measurements of its diameter with high dry magnification and the filar ocular micrometer. In the mature starfish egg the nucleus is of much smaller size than in the immature egg. Some of the eggs in every lot remain permanently immature and with care in selection immature eggs almost spherical in shape and with a spherical germinal vesicle may be secured.

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[1] Monne, L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1197.

Measurements of the swelling and shrinking of each egg were conducted separately. The egg was isolated, transferred to sea water diluted to the desired degree, and placed with a quantity of the diluted sea water in a small, closed glass chamber with optically plane faces. The temperature was about 21°C. but was not controlled. Measurements on cell and nucleus diameters were started as soon as possible after transfer to the diluted sea water, and were continued until both cell and nucleus reached an equi-

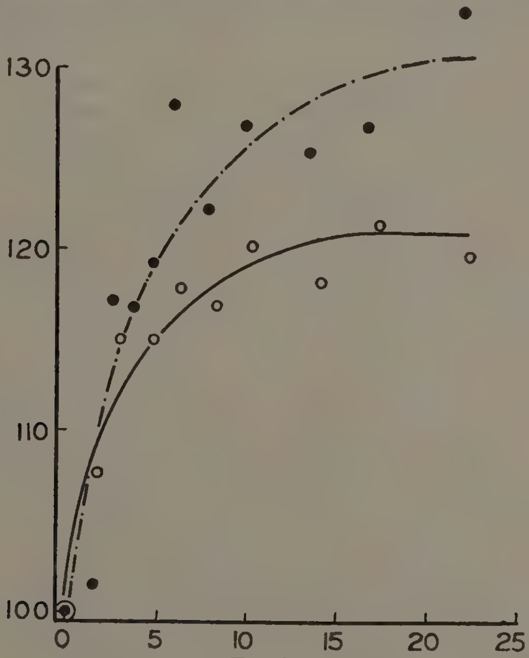

Fig. 1.

Comparison of swelling of cell and germinal vesicle of the immature starfish egg in 80% sea water. Abscissa represents time in minutes, and ordinate the percentage volume change. Open circles, cell; closed circles, germinal vesicle. Initial volume of cell 1,127,000 μ^3; initial volume of germinal vesicle, 160,300 μ^3.

librium volume, whereupon the egg was returned to 100% sea water and a new set of measurements made.

The changes in nuclear and cell volume secured for an egg placed in 80% sea water are shown in the accompanying graph, in which the average value of the equilibrium volumes attained in 100% sea water at the end of the experiment has been taken as the standard, and expressed as the 100% volume for both nucleus and cell. The advantage of this procedure is that it shows at a glance the greater percentage change in volume of the nucleus.

The inaccuracies of the present method are quite evident from the scattering of the points in the graph. However, the data shown and those secured in similar experiments demonstrate (a) that the nucleus swells and shrinks in the same sense as the cell does, and that the cell as a whole approaches an equilibrium volume more rapidly than does the nucleus (b) that the cell as a whole shows a smaller percentage increase in volume than does the nucleus (c) that both cell and nucleus shrink more rapidly than they swell. Finding (b) suggests that the germinal vesicle contains a smaller percentage of osmotically inactive material than does the cytoplasm. The more tardy approach to equilibrium volume by the nucleus is probably due in the main to the fact that the inwardly diffusing water must pass through the cytoplasm before it can enter the nucleus. Considerably more accurate data and a detailed mathematical treatment would be required to determine quantitatively the rôle played by the nuclear membrane in limiting the rate of passage of water between nucleus and cytoplasm.

Miscellaneous Manuscripts

8546 C

Chemotropism of Leucocytes in Relation to Their Rate of Locomotion.*

HAROLD M. DIXON AND MORTON McCUTCHEON.

From the Department of Pathology, University of Pennsylvania, School of Medicine.

A method was described[1, 2] for measuring the chemotropic reaction of polymorphonuclear leucocytes *in vitro*. Leucocytes were observed with the microscope and their paths recorded as they approached bacteria or other sources of attraction. In measuring the reaction to these attracting bodies only the direction of the path was taken into account, not the rate of locomotion. It seemed possible, however, that leucocytes might respond to such attraction not only by moving toward its source but by traveling at increased speed. In this case it would be desirable to take account both of direction and of velocity of approach in measuring the chemotropic reaction.

The point at issue has been touched on by previous observers,[3, 4, 5] who, however, did not make actual measurement to compare the rate of locomotion of leucocytes approaching a source of attraction with that of leucocytes moving at random. We have made such a comparison by means of a simple method which has been described by one of us.[6]

A minute clump of bacteria or of other particles is placed on a glass slide, where, after drying, it appears as a flat oval or circular body from 50 to 150 microns in diameter. A drop of blood, obtained by puncturing the finger, is placed on a coverslip, lowered on the bacterial mass and allowed to spread between slide and coverslip. The preparation is sealed with petrolatum and examined with the high power of the microscope in a warm box at 37°C.

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*This investigation was aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

[1] McCutcheon, M., Wartman, W. B., and Dixon, H. M., *Arch. Path.*, 1934, **17**, 607.

[2] Dixon, H. M., and McCutcheon, M., *Arch. Path.*, 1935, **19**, 679.

[3] Schade, H., and Mayr, K., *Krankheitsforschung*, 1930, **8**, 354.

[4] Schultz, E., *Z. exp. Med.*, 1932, **84**, 609.

[5] Philipsborn, E. v., *Fol. haematologica*, 1930, **43**, 142.

[6] McCutcheon, M., *Am. J. Physiol.*, 1923, **66**, 180.

After waiting half an hour for the cells to develop their normal rate of locomotion,[6] one edge of the bacterial clump is brought into view; the image of the microscopic field is projected on a piece of paper by means of a drawing ocular, and the position of each polymorphonuclear leucocyte is recorded at half-minute intervals for 5 minutes or until the cell moves out of the field. The total path traversed by each cell is measured in millimeters and converted into microns by multiplying by a factor that depends on the magnification. In this way the rate of locomotion is obtained in microns per minute.

Chemotropism is computed by measuring the net approach of cell to source of attraction; that is, the final distance of the cell from the bacteria is subtracted from the original distance; the quantity thus obtained is divided by the total path traversed in the same time. This measure of chemotropism is independent of the rate of locomotion. Values of chemotropism may range from +1.00, maximal positive, to —1.00, maximal negative chemotropism; most cells have intermediate values, 0.00 representing random movement. The calculation has been explained more fully elsewhere.[2] Leucocytes in the same microscopic field as the bacteria yield, with few exceptions, high positive values for chemotropism.

After recording the paths of cells close to the bacteria, a field several millimeters distant is observed in the same way, the rate of locomotion and value of chemotropism being determined for each cell. Since the duration of the experiment has been found to affect the rate of locomotion,[7] we have alternated observations between fields close to the bacteria and fields remote from them.

The following sources of attraction have been used: *Staph. albus*

TABLE I.

Rate of Locomotion and Chemotropism of Human Polymorphonuclear Leucocytes.

		Near Source of Attraction			Remote from Source of Attraction		
Source of Attraction	No. of Experiments	No. of Cells	Rate of Locomotion Microns/min.	Mean Value of Chemotropism	No. of Cells	Rate of Locomotion Microns/min.	Mean Value of Chemotropism
Staph. albus	4	27	28 ± 7.6	+.71	23	32 ± 7.4	—.06
Staph. aureus	5	63	32 ± 5.6	+.63	37	39 ± 5.3	+.06
Collodion	7	49	35 ± 8.2	+.55	44	35 ± 8.1	—.07

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[7] McCutcheon, M., *Am. J. Physiol.*, 1924, **69**, 279.

(from agar slants); *Staph. aureus* (also grown on agar but washed 3 times in distilled water); collodion particles (these particles, made by the method of Loeb,[8] are of about the size of bacteria; they were washed 3 times in distilled water). The results are given in the accompanying table. On the left side of the table are given values of chemotropism and rates of locomotion (with standard deviation) computed from the paths of cells in the same microscopic field as the source of attraction, while the values on the right side were obtained from cells remote from the source of attraction. The mean values for chemotropism are seen to be strongly positive in fields containing the source of attraction but approximate zero in remote fields; that is, in remote fields, leucocytes were moving at random. Comparing the rates of locomotion it is seen that in no case is the average rate of cells reacting chemotropically greater than that of cells moving at random. In the case of collodion particles the 2 rates are the same, 35 microns per minute; with *Staph. albus* the difference in rates is not significant, while with *Staph. aureus,* leucocytes in distant fields appear to have moved slightly faster than those near the bacteria (the reason for this is not clear). Thus our experiments give no evidence that cells reacting chemotropically travel faster than those moving at random.†

In interpreting these results, 3 possible sources of error have been held in mind. First, when bacteria are taken directly from the nutrient medium without being washed, they might carry with them sufficient salt to make hypertonic that part of the preparation immediately surrounding the bacteria. Consequently the rate of locomotion of leucocytes close to the bacteria might be reduced, thus neutralizing any tendency of attracted cells to move with increased velocity. This criticism would apply to the experiments with *Staph. albus* but not to those with the other particles, which were thoroughly washed with distilled water.

Second, the clump of bacteria or collodion might prevent slide and coverslip from coming as close together in the field of chemotropic attraction as elsewhere in the preparation. Such difference in thickness of the preparation might affect the rate of locomotion of leucocytes. Fortunately the arrangement of the erythrocytes, whether they occur singly, in twos or threes, or in rouleaux, gives an indication of the thickness of the preparation in any field. The rate of locomotion in thick and in thin fields was determined several times, and consistent differences were not found, except in fields so thick that they were obviously unsuitable for observation.

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[8] Loeb, J., *J. Gen. Physiol.*, 1922, 5, 109.

†Our conclusions do not necessarily conflict with those of Schulz,[4] who stated that increase in rate occurred only *just before* the leucocyte reached the bacteria.

Third, bacteria or other attracting bodies might give off substances toxic to leucocytes, reducing their rate of locomotion. Since the highest concentration of such substances would be in the zone immediately about the bacteria, any increase in velocity due to chemotropism might be masked by the toxic effect.

It appears impossible to rule out this objection entirely, but we have tried to meet it by using 3 different attractive bodies of which *Staph. albus* and thoroughly washed collodion particles would be expected not to prove toxic.

Summary and Conclusion. Quantitative evidence is presented that human polymorphonuclear leucocytes, when reacting to attraction by bacteria or other bodies *in vitro,* travel at no greater rate than do these cells when moving at random. It is therefore concluded that chemotropic response is one of direction only, while the rate of motion depends on such factors as temperature,[9] osmotic pressure of the medium, and on the internal condition of the cell.[5]

8547 C

Progestin Content of Human Placenta.

D. A. McGINTY, N. B. McCULLOUGH AND J. G. WOLTER.
From the Research Laboratories, Parke, Davis and Company, Detroit, Michigan.

The amount of progestational hormone in the human placenta is of interest not only because of contradictory reports as to the quantity present but because of the significance of this organ along with the corpus luteum as a source of luteal hormone during pregnancy.

Ehrhardt and Weigel[1] using implantation methods reported failure to demonstrate corpus luteum hormone in the human placenta. However, Ehrhardt[2] a few months later, using at this time placental extracts from which estrogenic fractions were separated, found as much as 10 (Clauberg) rabbit units in a single term placenta. Negative or weakly positive results were obtained in every instance when unseparated extracts were injected.

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[9] McCutcheon, M., *Am. J. Physiol.*, 1923, **66**, 185.

[1] Ehrhardt, K., and Weigel, W., *Endocrinol.*, 1933, **18**, 225.

[2] Ehrhardt, K., *Münch. Med. Woch.*, 1934, **81**, 869.

Adler, Fremery and Tausk[3] likewise demonstrated the presence of hormone in human placentas but only when estrogenic substances were removed. They reported, however, one rabbit unit in 500 to 1500 gm. fresh tissue or roughly one rabbit (Corner-Allen) unit per placenta.

Using the method of Corner and Allen for preparation of total placental extracts and that of Allen and Meyer for separation of estrogenic and progestational fractions, we have extracted two 10 pound lots of human full term placentas. Progestin content was assayed on 1000-1200 gm. rabbits following the McPhail[4] procedure. Four specimens from various regions of the uterus were sectioned, the average degree of proliferation being recorded. While the progestational proliferation at the smaller doses of placental extract was somewhat atypical, due undoubtedly to incomplete removal of estrogenic substances, moderately accurate estimates of activity were possible. In one of the 2 experiments, the estrogenic fraction was assayed. The results are shown in the table.

TABLE I.

Extract No.	Equivalent Fresh Placenta, gm.	Response
No. 350228 P	50	0 to +1
	100	+1
	150	+2 to +3
	200	+3
	250	+2 to +3
	300	+3
*No. 350228 PL	50	+1
	100	+1
	150	+2

*The estrin content of the estrogenic fraction of this extract assayed 3000 rat units per kilo placenta.

It will be noted from these results that in agreement with Ehrhardt fresh placenta contains approximately 7 units† progestational hormone per kilo of tissue and that significant progestational effects are seen with but the equivalent of 50 gm. fresh placenta. Using

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[3] Adler, A. A., Fremery, P., and Tausk, M., *Nature*, 1934, **133**, 293.

[4] McPhail, M. K., *J. Physiol.*, 1934, **83**, 145.

† The rabbit unit employed in this laboratory is based on the use of immature rabbits of average weight of 1100 gm. The unit is, therefore, somewhat larger than that of McPhail who used 750-950 gm. rabbits but about equal to the Clauberg unit, the latter being slightly less than one-half the Corner-Allen (adult rabbit) unit. The Corner-Allen unit is equivalent approximately to the international unit.

5 to 10 times the minimal amount of extract which will cause morphological changes, the degree of response did not exceed stage 3. This was interpreted as being due to the inhibitory effect of contaminating estrogenic hormone on progestin effect. Hisaw reports that 10 rat units of estrin will mask the progestational response of one rabbit unit progestin.

While it is evident that the human organism contains significant amounts of progestational hormone during pregnancy, there is present on the basis of present information relatively little during the normal ovarian cycle. Clauberg[5] reported atypical but definite progestational rabbit uterine proliferation with extract of human corpora lutea corresponding to 55 gm. fresh tissue. Pratt[6] obtained a positive reaction with the equivalent of 60 gm. fresh tissue in one experiment and 75 gm. in another, estrogenic substances being separated. Most of the corpora were from cases in the latter half of the intermenstruum. Thus from these scanty data there is present in the human (single mature corpus luteum) but .03 rabbit unit of hormone whereas a mature hog ovary may contain 30 times as much. Despite the small amount of progestin present in the nonpregnant human ovary as compared to that in ovaries of other species, no little significance may be attached to the importance of this hormone during pregnancy on that basis. While the corpus luteum may not be essential for continuance of human pregnancy, one cannot conclude that the luteal hormone is dispensable from that observation alone since the placenta may produce its own progestin.

8548 C

Renal Excretion of Cyanol in the Sculpin.

ALLAN L. GRAFFLIN. (Introduced by W. B. Cannon.)

From the Department of Anatomy, Harvard Medical School.

On the basis of previous work from his laboratory, Höber[1] postulated that cyanol would not be excreted by the aglomerular kidney, and obtained negative results in a few experiments on the goosefish. Marshall and Grafflin[2] reported that cyanol is not excreted by the aglomerular kidney of the toadfish at low dosage, but that at high dosage a small amount is excreted. They further found that when the sculpin, a glomerular marine teleost, was rendered functionally aglomerular by large doses of phlorizin, cyanol failed to appear in the urine. In view of Höber's negative results on the goosefish, the following experiment on this species (*Lophius piscatorius*) at rather high dosage is of interest. The specimen, weight 12 kilos, was given 1.5 gm. of cyanol intramuscularly, and 17 hours later showed a plasma concentration of 15.8 mg.%, urine 2.6 mg.%. A second urine taken an hour later contained 2.5 mg.%.

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[5] Clauberg, C., Thiel, H.-W., and Ziecker, R., *Arch. f. Gynäk.*, 1932, **152**, 61.

[6] Pratt, J. P., *Arch. Path.*, 1935, **19**, 380.

[1] Höber, R., *Pflüger's Arch.*, 1930, **224**, 72.

Four experiments upon the excretion of cyanol in the sculpin (*Myoxocephalus octodecimspinosus*) at rather high plasma levels are summarized below. The animals (167 to 305 gm.) received 40 mg. of the dye intramuscularly from 4 to 17 hours before the beginning of the urine collection period, which varied from 8½ to 17½ hours in length. Blood was drawn at the beginning and end of the period, and the mid-period concentration calculated. Plasma (P) and urinary (U) concentrations of cyanol are expressed as mg. %, urine flows as cc. per kg. per 24 hours. When the U/P ratios are compared with Clarke's[3] figures for xylose in the sculpin, it is apparent that glomerular filtration alone seems adequate to account for all of the cyanol excreted in the urine. This conclusion is reinforced by the more recent finding that xylose gives measures of the glomerular filtrate which are too low.

1. (P) 23.3, (U) 57.7, U/P ratio 2.4, flow 11.6 cc.
2. (P) 18.7, (U) 34.7, U/P ” 1.9, ” 17.0 ”
3. (P) 32.0, (U) 43.0, U/P ” 1.3, ” 25.5 ”
4. (P) 14.9, (U) 25.0, U/P ” 1.7, ” 46.5 ”

Two experiments in which glucose and cyanol were compared under phlorizin are particularly interesting in view of Cope's[4, 5] previous observations upon rabbits. In unphlorizinized animals he found the clearances of xylose and cyanol to be essentially identical in 10 out of 13 experiments. Under phlorizin, however, while the glucose clearance equalled that for xylose, the cyanol clearance dropped markedly and averaged only 42% of the xylose clearance. From our experiments it is clear that a similar depression of cyanol excretion under phlorizin occurs in the sculpin. The data upon one

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[2] Marshall, E. K., Jr., and Grafflin, A. L., *J. Cell. and Comp. Physiol.*, 1932, **1**, 161.

[3] Clarke, R. W., *J. Cell. and Comp. Physiol.*, 1934, **5**, 73.

[4] Cope, C. L., *J. Physiol.*, 1933, **80**, 238.

[5] Cope, C. L., *J. Physiol.*, 1933, **80**, 253.

TABLE I.

Sculpin No. 88; 475 gm.

	Glucose	Cyanol
Plasma	473	12.1
Urine	708	12.8
U/P ratio	1.50	1.06
Plasma	446	10.9
Urine	748	13.9
U/P ratio	1.68	1.28

animal are given in the accompanying table. This sculpin received glucose and cyanol intramuscularly on the day before the experiment, and 12 mg. of phlorizin (in the range of dosage recommended by Marshall and Grafflin) was administered intramuscularly 2½ hours before the first urine collection period was started. Blood samples were drawn at the beginning of the first and at the end of the second period, and the mid-period plasma concentrations calculated. The periods were approximately 3½ hours in length. Glucose and cyanol are expressed as mg.%. In this animal the cyanol clearance was in the first period 77%, and in the second period 76% of the simultaneous glucose clearance. In the second experiment the percentages were 75% and 67% in the 2 consecutive periods.

The interpretation of this depression of cyanol clearance under phlorizin has been discussed by Cope and no satisfactory explanation can yet be offered. It is striking that it holds true at both ends of the vertebrate scale: on the one hand in the rabbit, with its complex nephron, and on the other hand in the sculpin, whose nephron possesses, in addition to the glomerulus (and neck), only the analogue of the proximal convoluted tubule (brush border segment) of the mammalian nephron.

8549 C

dl-Methionine in Developmental Growth.*

Frederick S. Hammett and Kiveley Padis.
(Introduced by A. J. Goldforb.)
From the Research Institute of the Lankenau Hospital, Philadelphia.†

It is known that methionine can replace cystine as a growth essential in the diet of rodents.[1, 2, 3] This report extends to the invertebrate marine hydroid *Obelia geniculata* the premise that this sulfur-containing amino acid is a growth stimulant. The purpose is to see whether lower as well as higher organisms can utilize —S— sulfur for growth purposes, and whether any particular phase of growth activity is responsive thereto, since this point is unsettled in work with rodents.

Through the courtesy of Professor du Vigneaud we obtained enough dl-methionine for 32 experiments. In these the developmental growth of some 6000 animals in methionine cultures was compared with that of a like number in plain sea water. Description of the organism and its growth together with that of the experimental and other procedures is given in detail elsewhere.[4, 5] Tests and controls were run simultaneously under like conditions of temperature, illumination, and pH. Results are tabulated as condensed consistency data from which is seen the trend of test deviation from control in expression of new growth initiation, proliferation, differentiation, and organization, as well as that for maintenance, regression, and catabolism.[6] The table also gives the concentrations used and the number of experiments, colonies, hydranths, and gonophores. Since general reaction was essentially the same in the stated concentrations the data were combined into one set of figures to save space. Those for the 6 experiments at

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*Aided by a grant from the International Cancer Foundation.

†The work was done at the Marine Experimental Station of the Institute, North Truro, Massachusetts, with the technical assistance of Misses Chatalbash, Elliott, and Coward, and Dr. Nicholas Padis.

[1] Jackson, R. W., and Bloch, R. J., *J. Biol. Chem.*, 1932, **98**, 465.

[2] Weichselbaum, T. E., *et al.*, *Nature*, 1932, **129**, 795.

[3] du Vigneaud, V., Dyer, H. M., and Harmon, J., *J. Biol. Chem.*, 1933, **101**, 719.

[4] Hammett, F. S., *Protoplasma*, 1933, **7**, 297.

[5] Hammett, F. S., *Protoplasma*, 1935, **23**, 326.

[6] Lavine, T. F., *Am. J. Cancer*, 1935, **25**, 809.

TABLE I.

Trend of Test Deviation from Control with Respect to Developmental and Metabolic Activity in dl-Methionine Cultures.

''S''		$8x10^{-8}$		$1.6x10^{-7}$	$3.2x10^{-7}$	
M/1000x		400(7)		200(10)	100(9)	
	+	—	0		Control	Test
New Growths	10	5	6	No. Experiments	26	
Proliferation	9	6	9	'' Colonies	326	321
Differentiation	9	10	10	'' Hydranths	5247	5240
Organization	7	8	9	'' Gonophores	368	330
Maintenance	5	20	1	% Regression	27	30
Catabolism	8	16	2	% Catabolism	93	86

M/50,000 and M/25,000 were omitted because they show nothing but toxicity.

The table shows methionine acts to stimulate growth in obelia as in rodents but that the early stages, not the later, are influenced. Thus both new growth production and proliferation were enhanced while the succeeding phases of differentiation and organization were essentially unaffected. The evidence is the fact that test progression was greater than control in 19 and less in but 11 of the combined expressions of the early activities, while it was greater than control in but 16 and less in 18 of the later. The fact that catabolic disintegration was less in tests than controls is consistent.

The demonstration that it is growth in which cell increase in number plays a major part that was differentially enhanced is significant. For since it is the —SH group which appears to be specifically effective in this direction[7] these findings support the possibility that methionine is converted first to cystine[1] and then to cysteine.[8, 9]

Probably consistent with the foregoing is the fact that somewhat higher concentrations were effective and toxic with sulfur as —S— than with sulfur as —SH. This would be expected if conversion preceded activity.

Finally it is recorded that cystine increases respiratory metabolic activity[10] and our results show that such occurred with methionine. Maintenance was less and regression increased.[5] Whether this be expression of specific dynamic effect or not, a matter which should be studied with higher organisms, the correlation supports the assumption of conversion.

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[7] Hammett, F. S., *Protoplasma*, 1930, **11**, 382.

[8] Loring, H. S., Dortmann, R., and du Vigneaud, V., *J. Biol. Chem.*, 1933, **103**, 399.

[9] Lewis, H. B., *Physiol. Rev.*, 1924, **4**, 394.

[10] Miyao, S., *Gann*, 1935, **29**, 10.

Summary and Conclusion: Thirty-two experiments with nearly 12,000 obelia, half of which were exposed to dl-methionine in varied concentration showed this sulfur containing amino acid acts as a growth stimulant to this lowly animal as it does to higher. The effect was differentially produced on growth in which cell increase in number was dominant. The results taken with correlated data are consistent with the assumption that methionine may be converted to cystine in the living organism.

8550 C

The Action of Ergometrinine.

K. K. CHEN, EDWARD E. SWANSON, AND CHESTER C. HARGREAVES.
From the Lilly Research Laboratories, Indianapolis.

Ergometrinine is one of the newer alkaloids of ergot isolated by Smith and Timmis.[1] It decomposes at about 195° and has an optical rotation of $[\alpha]^{20}_{5461} + 598°$. Its empirical formula is the same as that for ergometrine, that is, $C_{19}H_{23}O_2N_2$. Raymond-Hamet[2] reported that in the dog ergometrinine caused a rise of blood pressure and vasoconstriction, and that with a dose of 24.5 mg. per kg., it abolished the vasoconstricting action of adrenalin. He concluded that ergometrinine was weaker than ergometrine.

With the aid of Mr. Howard B. Fonda, Experimental Research Laboratories, Burroughs Wellcome and Company, Tuckahoe, we were able to secure 50 mg. of ergometrinine nitrate. The material is easily soluble in water and exhibits a blue fluorescence in aqueous solution. Unlike ergotoxine or ergotamine, ergometrinine does not inhibit the adrenalin response on the isolated rabbit's uterus in a concentration as strong as 1:27,777 (the Broom-Clark test[3]), but on the contrary, it exerts a weak stimulating action itself. When assayed by the method described previously,[4] ergometrinine HNO_3 was shown to be 1/100 as active as ergotocin maleate in 6 observations (Fig. 1A). Since the results obtained in our laboratory indi-

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[1] Smith, S., and Timmis, G. M., *Nature*, 1935, **136**, 259.

[2] Raymond-Hamet, *Compt. rend. Soc. de biol.*, 1935, **120**, 1208.

[3] Broom, W. A., and Clark, A. J., *J. Pharm. and Exp. Therap.*, 1923, **22**, 59.

[4] Swanson, E. E., Hargreaves, C. C., and Chen, K. K., *J. Am. Pharm. A.*, 1935, **24**, 835.

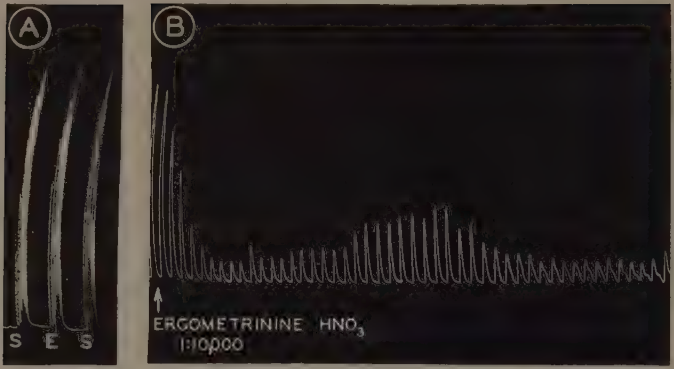

FIG. 1.

A. Assay of Ergometrinine on Isolated Rabbit's Uterus. At S 0.03 mg. of ergotocin maleate was added. At E 3 mg. of ergometrinine HNO_3 were applied. There was a "washout" after each response.

B. Action of Ergometrinine on Isolated Rabbit's Intestines. Note the inhibition after the introduction of ergometrinine HNO_3.

cate that ergotocin has identically the same pharmacological effect,[5] the ratio of activity between ergometrinine and ergometrine must be also 1:100. The new base was found to possess a similarly low potency on the cock's comb—only about 0.7% of the activity of ergotocin maleate according to the U.S.P. procedures,[6] a total of 19 birds being used. Colorimetrically,[7,8] however, ergometrinine HNO_3 proved to have a value of 106% as compared with ergotocin maleate. It relaxed isolated rabbit's intestines (Fig. 1B). By local application of a one per cent solution, it did not dilate the rabbit's pupil. An intravenous injection of ergometrinine HNO_3, 4 mg. per kg., in an etherized cat was followed by a prolonged fall of blood pressure with marked changes of the electrocardiogram. As shown in Fig. 2, there occurred bundle branch block and nodal tachycardia during the lowering of blood pressure, and a typical nodal rhythm as soon as the pressure rose to the initial level.

Summary. Ergometrinine has approximately 1/100 the oxytocic activity of ergotocin (or ergometrine). It inhibits isolated rabbit's intestines. In a cat, a dose of 4 mg. per kg. caused a fall of blood pressure accompanied by bundle branch block, nodal tachycardia, and finally nodal rhythm.

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[5] Chen, K. K., Swanson, E. E., Kleiderer, E. C., and Clowes, G. H. A., *J. Pharm. and Exper. Therap.* (In press).

[6] Pharmacopœia of the United States, 11th rev., 1936, 148.

[7] Van Urk, H. W., *Pharm. Weekblad,* 1929, **66**, 473.

[8] Smith, M. I., *Pub. Health Rep.,* 1930, **45**, 1466.

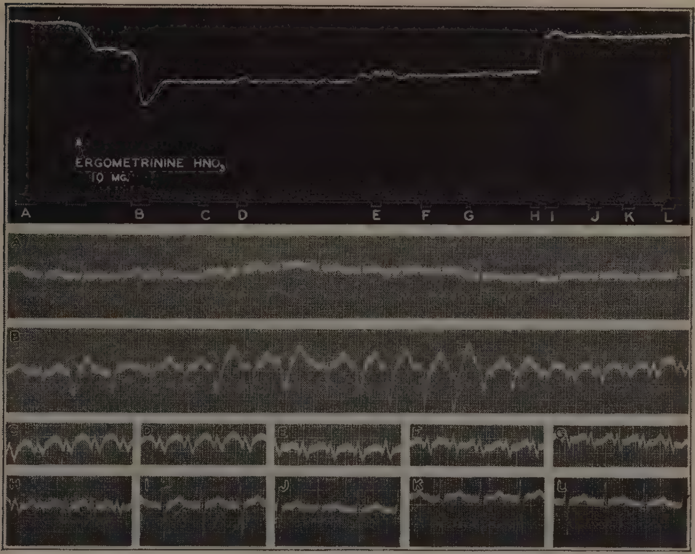

Fig. 2.

Action of Ergometrinine on Blood Pressure and Electrocardiogram. Cat No. 1372, female, weighing 2.5 kg., was anesthetized with ether. The letters marked on the electrocardiograms correspond to those on the kymographic tracing which shows from above down the carotid pressure, time, and the base line.

We are indebted to Messrs. C. Earl Powell, Robert C. Anderson, and Asa N. Stevens for their valuable assistance in this work.

8551 C

Possible Bipolar Nuclear Distribution in Bacteria.

James W. Brooke. (Introduced by H. J. Sears.)

From the Department of Bacteriology, University of Oregon Medical School, Portland.

Variations in the staining intensity of portions of the bacterial cell so that the bacterium appeared to contain granules situated at the poles were noted while employing a Gray's flagella stain on a glucose broth culture of *Eberthella typhi.* Subsequent investigation demonstrated that with improved technic the granules could be shown in a variety of bacteria irrespective of the sugar employed but inversely related in size and frequency of appearance to the age of the culture.

A slide is prepared as for a flagella stain and a ring marked on it with a wax pencil. Two loopfuls of sterile water are placed within the ring and to this is added a loopful of the culture. This is allowed to dry without heating and a quantity of 10% alcoholic basic fuchsin sufficient to cover completely the ringed area is dropped on. After 7 to 10 minutes, during which it is kept moist with drops of 95% alcohol, the stain is washed off with water, a drop of acid alcohol placed at the periphery of the ringed area, and instantly washed off with a gentle stream of water. After drying in the air, the slide is ready for observation.

Granules were definitely demonstrated in: *Eberthella typhi, Proteus vulgaris, Escherichia coli mutabile, Shigella paradysenteriae,* Flexner (2 strains), *Sh. paradysenteriae,* Sonne, *Salmonella paratyphi, Pseudomonas fluorescens, Alcaligenes fecalis, Alc. bronchisepticus.*

Granules were apparent in *P. vulgaris* when cultivated in broth containing glucose, salicin, maltose, sucrose, mannite or lactose. At the 96-hour stage of growth the salicin culture failed to show the granules and at the 120-hour stage the mannite exhibited them but poorly. Lactose broth cultures gave poor results at the 144-hour stage and at 168 hours only glucose and maltose cultures showed the granules. At 254 hours no granules were demonstrable from any medium though viability was shown by growth on transplanting. The granules did not appear when the organism was cultured on meat-extract agar, meat-extract or meat-infusion broth, Dorsett's egg medium or glycolized blood agar. Sugar-free agar slants of *Alc. fecalis* and *bronchisepticus* did not show the staining reaction in contradistinction to lactose broth cultures of the same organisms. On an Endo plate the Sonne dysentery bacillus showed no granules while a strain of Flexner type of paradysentery bacillus did. On culturing in lactose broth the Sonne organism showed faint but perceptibly stained polar areas. An agar slant of *P. vulgaris* did not manifest the staining reaction but a maltose broth suspension which was allowed to stand overnight in the ice-box contained a few granular staining organisms. A stain made immediately after the suspension had been prepared did not show the granules. *B. cloacae, Br. abortus,* and 2 strains of *Sh. dysenteriae* did not show the granules with the technic employed. Two Gram-positive organisms, *B. mesentericus* and *B. subtilis,* proved granule-negative.

When a maltose broth culture of *P. vulgaris* was washed by centrifugalization with 95% alcohol, 70% alcohol, N/10 HC1, $CHC1_3$, or 0.85% NaCl no granules could be demonstrated. Upon similar treatment with distilled water positive results were obtained. A control stain made with the original culture showed granular staining in approximately 70% of the bacteria encountered in a random field.

When 5 cc. portions of a maltose broth culture were treated with 5 cc. of 5% NaCl, 5% phenol, and 2% tincture of iodine respectively and allowed to stand 24 hours, the characteristic staining was noted in the first instance only. That this was related apparently to the viability of the organism was shown by the fact that the NaCl culture was the only one viable. Fischer[1] reports similar staining of bacteria plasmolyzed with 2.5% NaCl solution. Such preparations responded only to 0.1% basic fuchsin among the ordinary simple stains.

Methylene blue, crystal violet, carbol fuchsin, 0.1% basic fuchsin, and malachite green stains employed on maltose broth cultures of *P. vulgaris* did not yield the granule-staining results yielded by 10% basic fuchsin.

In an attempt to ascertain the chemical nature of these deeply staining areas, iodine, Scharlach R, and the polychromatic methylene blue stains were employed. By none of these were they demonstrable and the assumption that these granules were neither starch, glycogen, fat nor volutin seemed justified. On the basis of a negative Millon's reaction and failure to reduce nitric acid, it was tentatively assumed that they contained no hydroxyphenyl proteins and were of non-reducing nature.

The granular staining was perceptible with the iron-hematoxylin technic on a maltose broth culture of *P. vulgaris.*

The presence of a sugar appears essential for the demonstration of these granules. Utilization of the sugar does not seem to be necessary as *P. vulgaris* does not utilize either mannite or salicin. These sugars are the first to lose their granulogenic powers, however. The fact that the granules can be demonstrated in a sugar broth suspension from an agar slant after standing overnight in a temperature not conducive to growth would suggest a physico-chemical relationship. It may be that the sugar molecule passes through the cellular membrane to activate the existing but unstainable granule in such a manner that it becomes receptive to the stain. Since the granules are demonstrable with iron-hematoxylin and strong basic fuchsin, both of which are nuclear stains, the assumption that they may be composed of nuclear material does not appear to be entirely unwarranted. Zettnow[2] based his classification of granular inclusions on staining reactions and according to his theory, these polar granules would fall into the category of chromatin. Since they do not appear in the killed cultures, it would seem that they are seen only in the living organism. This agrees also with their gradual disappearance from an aging culture. It may be that they are terminal concentrations of nuclear material just previous to fission and that this nuclear material is in some manner rendered especially permeable and susceptible to the chromatin stains. Considering the regularity of their appearance under certain conditions and not under others, the possibility that they may be artifacts would seem to be excluded.

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[1] Fischer, "Structure and Functions of Bacteria," Oxford, 1900.

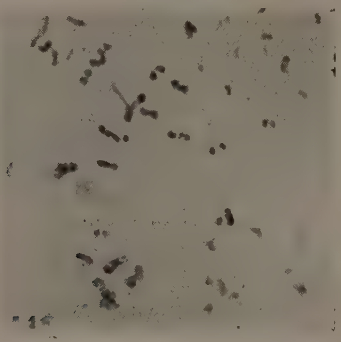

FIG. 1.
48-hour maltose broth culture *P. vulgaris* showing granules.

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[2] Zettnow, *Z. f. Hygiene*, **85**, 17.

8552 P

Water Filtration by the Oyster and a New Hormone Effect upon the Rate of Flow.

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Estimates of water passing through an oyster based on: (1) amount of sediment deposited; (2) number of food organisms collected; and, (3) direct measurement of flow through a tube in the cloacal chamber, vary from 333 cc. per hour to 200 quarts per day. Galtsoff[1] with method (3) obtains maximum pumpage of 7 liters per hour. This method is defective since tube in the cloacal chamber prevents normal operation of the first and second zones of control; the shell and the reflected mantle borders, and interferes with blood supply to the gills from the accessory hearts, Hopkins.[2] In *Ostrea virginica* much of the water entering the right gill anterior to the adductor muscle leaves through an asymmetric excurrent aperture on the right side hence never enters the cloacal chamber. The small tube, 6 mm. creates high ratio of friction to hydraulic head developed.

A superior method, Nelson,[3] measuring all water passed while the oyster functions normally, is attachment of the rubber apron of Moore[4] to a large (23 mm.) tube into the exhaust chamber of the constant level tank of Galtsoff.[1] Using this method a head of 7 mm. of water was developed by an oyster 11.8 x 7.8 cm. at 25.5°C., as compared with 4 mm. previously obtained. Little water passes below 10°C. but above this temperature pumpage rises rapidly to maximum of 26 liters per hour in an *O. virginica* 11.5 x 8.9 cm. A total of 111 liters was filtered in 7 hours at 24-27°C. Above 30° flow decreases sharply from partial closure of shell and reflected mantle borders. Hopkins[2] shows similar effects in *O. gigas* above 20.°

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[1] Galtsoff, P. S., *Science*, 1926, **63**, 233; *J. Gen. Physiol.*, 1928, **11**, 415; *Bull. U. S. Bur. Fish.*, 1928, **44**, 1; 1935, **47**, 143; *Proc. Nat. Acad. Sci.*, 1930, **16**, 555; *Am. J. Pub. Health*, 1936 (in press).

[2] Hopkins, A. E., *J. Exp. Zool.*, 1933, **64**, 469; *Science*, 1934, **80**, 411; *Biol. Bull.*, 1934, **67**, 346; *J. Exp. Zool.*, 1935, **71**, 195.

[3] Nelson, T. C., *Anat. Rec.*, 1935, Supl. 68; *Bull. 351, N. J. Agr. Exp. Sta.*, 1921, **11**; *Rpt. N. J. Agr. Exp. Sta.*, 1922, 296; PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 90.

[4] Moore, H. F., *Bull. U. S. Bur. Fish.*, 1908, **27**, 1297.

Marked fluctions in rate occur independent of temperature, (Nelson,[3] Hopkins[2]). Flow is increased during active shell secretion and by addition of fresh oyster sperm to incurrent water of the male oyster. Sperm is effective in less than one minute, causing ostia to enlarge, permitting more rapid flow. A typical increase is from average flow of 5.9 liters per hour to 11. Initial rise in rate is followed quickly by marked decrease, succeeded by secondary rise to new high level, the duration of which depends on amount of sperm introduced. No response occurs in female oyster unless induced to spawn, in which case flow is markedly reduced owing to reversal of water passage through the ostia associated with expulsion of the eggs, Elsey[4], Galtsoff (unpublished observations).

Since spawning in the oyster is a group response, (Nelson,[5] Galtsoff[1]), increased rate of water flow in the males permits wider and more rapid dissemination of sperm. With its power to stimulate spawning in both females and males, (Galtsoff[1]), the group reaction is more definitely assured, especially where the animals are scattered. Reduced filtration by females during spawning greatly decreases capture of eggs by gills of the parent.

8553 P

Effect of Deuterium Oxide on Action of Some Enzymes.

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Since the discovery of heavy water much speculation of very diverse character has been advanced in regard to its possible effects on life. The most extreme views are presented by those who claim, without adequate experimental proof, that deuterium oxide is a most powerful destructive physiological agent and those who, basing their conclusions on experiments made with deuterium oxide in dilute concentrations, state that its pharmacological effects are of negligible character.[1, 2, 3] The authors have recently dis-covered an interesting and striking contrast in the biophysical and biochemical behavior of H_2O and D_2O, which consists of a difference in velocity of reaction shown by certain animal and vegetable enzymes suspended therein. Studies were made on the behavior of muscle oxydase from the rat, of oxidative enzymes of fresh, blood-free brain tissue from the cat, and of reductase of finely ground seeds of *Lupinus albus* by the Thunberg method of determining the rapidity with which a standard buffered solution of methylene blue is decolorized in special vacuum tubes. Over 50 experiments were performed. The method employed is described in full elsewhere.[4, 5] A marked difference was found to exist between identical enzymes suspended in H_2O and in the same kind of water to which small quantities of deuterium oxide had been added. This difference was demonstrated not only with concentrations of D_2O, 1:100, but also with concentrations of 1:2000 and less.

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[5] Elsey, C. R., *Trans. Roy. Soc. Can.*, 1935, Sec. V, 131, 142.

[1] Meyer, *J. Tenn. Acad. Science*, 1934, **9**, 225.

[2] Meyer, *J. Tenn. Acad. Science*, 1935, **10**, 111.

[3] Macht and Davis, *J. Am. Chem. Soc.*, 1934, **56**, 246.

Further studies were made in connection with the influence of deuterium oxide on the activity of another enzyme, catalase. Catalase from fresh rat muscle and from *Lupinus albus* seeds in distilled water and in water to which small quantities of deuterium oxide were added also revealed a difference in the speed of evolution of oxygen gas from hydrogen peroxide in the presence of these enzymes. The method employed was a modification of that described by Baldwin.[6] Briefly, it consisted of the use of a Thunberg tube into which were introduced one fifth molar of disodium phosphate and one fifth molar of diacid potassium phosphate. When the experiment is begun, the catalase suspension is allowed to run into the Thunberg tube and the oxygen gas evolved is collected by

TABLE I.
Experiments with Muscle Oxydase.
Technique: 2 cc. of muscle suspension plus 1 cc. of Thunberg solution introduced into tubes on water bath at 38° C.

Oxydase Prepared with H_2O Saline.		Oxydase Prepared with D_2O Saline, 1:100	
Tube	Decolorization Time	Tube	Decolorization Time
	min.		min.
1	180	1	149
2	186	2	120
3	182	3	115
4	180	4	140
Aver.	182	Aver.	131

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[4] Macht and Bryan, *J. Biol. Chem.*, 1935, **110**, 101.

[5] Macht, *Arch. internat. de pharmacodyn. et de therap.*, 1934, **49**, 175.

[6] Baldwin, *Am. J. Bot.*, 1935, **22**, 635.

displacement of water in a fermentation tube connected with the side arm of the apparatus. Tables I, II, and III show the character of the findings obtained in the numerous experiments performed.

TABLE II.
Experiments with Brain Tissue Extract.

Oxydase Prepared with H_2O Saline (NaCl, 0.9%)							Oxydase Prepared with D_2O Saline, 1:2000				
Tube	Decolorized in						Tube	Decolorized in			
	hr.	%	hr.	%	hr.	%		hr.	%	hr.	%
1	1	45	4	75	5½	100	1	1	55	4	100
2	1	45	4	75	5	100	2	1	55	4	100
3	1	45	4	75	5½	100	3	1	55	4¼	100

TABLE III.
Experiments with Catalase of *Lupinus albus*.
Technique: Placed in tube 1 cc. of H_2O, 3 cc. of H_2O_2 (3%), 1 cc. of KH_2PO_4 (M/5), and 1 cc. of Na_2HPO_4 (M/5).

	Series 1 (2 experiments) 0.5 cc. bean extract made with H_2O	Series 2 (2 experiments) 0.5 cc. bean extract made with D_2O, 1:100
Aver. Oxygen 15 min.	3.3 cc.	5.55 cc.

It will be noted that the enzymatic activity was accelerated in the presence of deuterium. In some of the experiments the enzymes exhibited a diphasic activity. This was especially true of more concentrated solutions of D_2O. There was a primary retardation, which was followed by a secondary and more marked acceleration. In all the experiments, however, a definite difference in reaction was noted between H_2O and D_2O media.

These findings have not only a purely theoretical scientific interest but also a practical bearing. It is well known that minute quantities of powerful drugs or poisons are often more readily detected by physiological tests on living animal or plant preparations *in vitro* than by chemical or even physical means.[7, 8, 9, 10] When studying such physiological reactions it is necessary to employ aqueous solutions of definite composition. Minute solutions of organic or inorganic impurities present in water used for physiological tests may profoundly influence the biological reactions. The writers' work indicates that even in case of pure water differences may occur in the physiological, pharmacological or biochemical responses due to variations in the quantity of the hydrogen isotope,

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[7] Hunt, *Science*, 1930, **72**, 526.

[8] Macht and Anderson, *J. Am. Chem. Soc.*, 1927, **49**, 2017.

[9] Hatcher, *American Druggist*, 1929, **79**, 22.

[10] Macht, *Am. J. Ophthalmol.*, 1931, **14**, 726.

deuterium, present in the sample used. When we recall that there are 2 known isotopes of hydrogen—namely, deuterium and tritium—and that 3 varieties of oxygen, differing slightly in their atomic weight, have been established, the subject appears to be even more complex than we at first suspected.*

8554 C

Assays of Hypertrophied Prostatic Tissue and of Urine for Estrogenic Substances.

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A high content of oestrin has been reported in normal ovarian tissues[1] and in tumors of the ovary,[2] breast,[3] and uterus.[4] Estrogenic substances have been demonstrated in the blood and urine, and in some cases a fairly high yield has been reported from male urine.

High yields of anterior-pituitary-like hormone have been obtained from urine in cases of teratoma of the testis,[5] chorio-epithelioma and hydatidiform mole,[5, 6] but Smith and Smith[7] have reported low levels of oestrin in such cases.

There have also been reports of atypical growth of prostatic

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*Since this paper was written there has appeared in these PROCEEDINGS (1936, **33**, 266) an article by Fox and Craig concerning the effect of deuterium on the hydrolysis of starch. This biological reaction was found to correspond qualitatively with those described in the foregoing paper.

†This work has been supported by a grant from the Anna Fuller Fund to the Department of Anatomy.

Acknowledgment is made for supervision of the biochemical work to Dr. Jack M. Curtis, National Research Council Fellow working in this Department as biochemist on endocrine problems.

[1] Allen, E., Pratt, J. P., and Doisy, E. A., *J. A. M. A.*, 1925, **85**, 399.

[2] Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1204.

[3] Lewis, D., and Geschickter, C. F., *J. A. M. A.*, 1934, **103**, 1212.

[4] Lewis, D., and Geschickter, C. F., *J. A. M. A.*, 1935, **104**, 45.

[5] Ferguson, R. S., *Am. J. Cancer*, 1933, **18**, 269.

[6] Geschickter, C. F., Lewis, D., and Hartman, C. G., *Am. J. Cancer*, 1934, **21**, 828.

[7] Smith, G. van S., and Smith, O. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 847.

tissues in rodents[8, 9, 10] and monkeys[11, 12] following injections of estrogenic substances, and some of the female organs, rudimentary in the male, have been induced to hypertrophy by injections of oestrin.[13]

The present study reports uniformly negative results from assays of hypertrophied prostatic tissues removed at operation from patients 60 to 80 years of age. Seven hypertrophied prostates, histologically hyperplastic but without evidence of malignancy, ranging from 9 to 28 gm., and also 23 samples of urine from 12 patients before and after prostatectomy, ranging in quantity from 1 to 15 liters, have given uniformly negative results in more than 100 tests. The methods used for extraction and assay of oestrin from tissue and from urine were those in current use for the detection of small amounts of this active substance.

These results indicate that oestrogenic substances were not present in significant quantities in either the urine or hypertrophied prostatic tissue examined.

8555 C

Enzymic Digestion of Lactalbumin Versus Casein *in Vitro*.*

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Since the experimental evidence concerning the nutritive value of lactalbumin is conflicting, an intensive study has been initiated in this laboratory to investigate the biological value of this protein from the standpoint of digestion, metabolism, and growth. Casein (being a protein of indisputable excellent biological value) was used as a standard of comparison.

In this report a summary is given of the results of tryptic-ereptic digestion of lactalbumin versus casein *in vitro*. The casein was

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[8] Lacassagne, A., *C. R. Soc. Biol.*, 1933, **113**, 590.

[9] Burrows, H., *Brit. J. Surg.*, 1934, **21**, 507.

[10] Burrows, H., and Kennaway, N. M., *Am. J. Cancer*, 1934, **20**, 48.

[11] Van Wagenen, G., *Anat. Rec.*, 1935, **63**, 387.

[12] Parkes, A. S., and Zuckerman, S., *Proc. Physiol. Soc., J. Physiol.*, 1935, **84**.

[13] Willier, B. H., *Anat. Rec.* (Suppl.), 1935, **61**, 50.

* Research paper No. 383, Journal Series, University of Arkansas, Fayetteville.

furnished by Merck and prepared according to the method of Hammersten. The lactalbumin was obtained from Harris Laboratories which is the purest product obtainable with a minimal ash content.

The technique employed has been described.[1] The proteins were studied in 3 concentrations, 120, 180, and 210 mg. As a source of trypsin and erepsin pancreatic and intestinal extracts were taken from rats of our colony stock diet, on which ration excellent growth is obtained.

A 6% protein solution (pH. 7.8), using approximately .08 N NaOH as a solvent, was prepared. An aliquot of this solution (1 cc. corresponding to 60 mg. protein); 20 cc. of boric acid buffer (pH 7.8); 1 cc. of pancreatic extract equivalent to 25 mg. pancreas; 2 cc. of intestinal extract equivalent to 100 mg. intestines, were made up in a 60 cc. Erlenmeyer flask to a final volume of 50 cc. with distilled water, alkaline to cresol red. The incubation

TABLE I.
Summary of Tryptic-ereptic Digestion of Casein and Lactalbumin.
1 cc. pancreatic extract. 2 cc. intestinal extract.

Hrs. of Incubation	Amt. and type of proteins	No. of samples	Mean hydrolysis in % total amino N	Difference between means of casein and lactalbumin in % total amino N	Significant Ratio
1	A	11	22.1 ± .80		
1	B	11	14.0 ± .93	7.1 ± 1.23	5.7
4	A	11	33.9 ± 1.14		
4	B	11	30.5 ± 1.79	3.4 ± 2.12	1.65
24	A	11	52.4 ± 1.32		
24	B	11	50.5 ± .87	1.90 ± 1.59	1.20
1	C	14	23.4 ± .84		
1	D	14	18.0 ± .82	5.4 ± 1.17	4.60
4	C	14	34.6 ± .72		
4	D	14	29.2 ± 1.15	5.4 ± 1.36	4.0
24	C	14	52.4 ± .95		
24	D	14	50.2 ± .94	2.2 ± 1.33	1.65
1	E	12	14.5 ± .69		
1	F	12	11.7 ± .41	2.8 ± .81	3.4
4	E	12	23.3 ± .79		
4	F	12	17.8 ± .85	5.5 ± 1.16	4.7
24	E	12	47.7 ± 1.23		
24	F	12	41.6 ± 1.09	6.1 ± 1.23	4.9

A = 120 mg. casein.
B = 120 " lactalbumin.
C = 180 " casein.
D = 180 " lactalbumin.
E = 210 " casein.
F = 210 " lactalbumin.

Significant Ratio:
2.5 to 2.8 approaching significance.
2.8 to 3.0 significance.
above 3.0 is high significance.

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[1] Sure, B., Kik, M. C., and Buchanan, K. S., *J. Biol. Chem.*, 1935, **108**, 11.

was allowed to proceed at 37.5°C for a maximum period of 24 hours. After an interval of one, 4, and 24 hours the amino nitrogen was determined in aliquots by means of the Van Slyke amino nitrogen apparatus. A blank contained the same as the sample, except that the tissue extracts were used after boiling for 10 minutes which destroyed trypsin and erepsin.

The results were expressed in percentage of total amino nitrogen, which was determined by acid hydrolysis. The total amino nitrogen for casein was 9.75% and for lactalbumin 10.7%.

A summary of the final average results is submitted in Table I. It will be noted from the speed of liberation of amino nitrogen that a significantly better digestion was observed with casein than with lactalbumin after the first hour incubation with all 3 concentrations of protein. There is also a noteworthy increase of digestion of casein after the fourth hour with 180 mg. protein. In the case of 210 mg. protein, there is a constant increase of amino nitrogen liberation in casein versus lactalbumin throughout the 24 hours of digestion.

It is, therefore, concluded that lactalbumin is less readily digested than casein *in vitro* by trypsin and erepsin when the incubation of these proteins is carried out under standard and comparable conditions.

8556 P

Segmental Differentiation in the Proximal Convoluted Tubule of the Mammalian Nephron.

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Edwards,[1] in discussing the proximal convoluted tubule of the mammalian nephron, refers to "its functional differentiation as manifest segmentally unaccompanied by obvious cytological change." Edwards and Schnitter[2] state that the proximal convoluted tubule of the mammal "is cytologically uniform throughout its length." In his most recent publication, Edwards[3] mentions the "unisegmental appearance" of the mammalian proximal

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[1] Edwards, J. G., *Anat. Rec.*, 1933, **55**, 343.

[2] Edwards, J. G., assisted by C. Schnitter, *Am. J. Anat.*, 1933, **53**, 55.

[3] Edwards, J. G., *Anat. Rec.*, 1935, **63**, 263.

convolution, and states that "it is probable that the obvious presence of definite segments in the proximal convolution of the renal tubule in fish has been cytologically concealed but functionally retained in this convolution in the kidney of other vertebrates."

Contrary to the statements of Edwards, convincing evidence of at least a bisegmental differentiation of the mammalian proximal convoluted tubule on cytological grounds was presented 25 years ago by Zimmermann.[4] In the dog and cat he found, in the pars recta, an abrupt transition from an epithelium with markedly irregular cell outlines (to be designated here as first segment) to one with sharp, rectilinear cell outlines, in general hexagonal in shape (to be designated here as second segment). In the cat the cells of the first segment showed abundant vacuoles (fat), while the second segment was entirely free of them. In the dog, on the other hand, just the opposite was true, the vacuoles being confined to the second segment. The striking difference, with respect to fat storage, in the terminal portion of the proximal convoluted tubule in these two animals had been described previously by Peter.[5] In his study of the cat Modell[6] missed the fat-free terminal portion entirely, and has been rightly criticized by Nakamura.[7]

An investigation of one aspect of this problem, namely, quantitative data upon the relative lengths of the two segments of the proximal convoluted tubule, is reported here. Four cats were killed instantaneously and their kidneys fixed in formalin. Small blocks were macerated in hydrochloric acid and then stained with Scharlach R or Sudan III. From each animal 15 proximal convoluted tubules were teased, mounted, drawn and measured. The relative lengths of the 2 segments were determined on the basis of fat content, the second segment being free of fat, as described above. The results are summarized in the accompanying table, in which the ranges of length (expressed in millimeters) and percentage are given for the 15 tubules studied from each specimen.

TABLE I.

Cat. No.	Total length Prox. C.T.	Length of 1st segment	Length of 2nd segment	2nd Seg.—% of total length
4	8.83-13.22	6.61-11.89	1.33-2.90	13.3-25.0
5	11.42-16.10	9.62-13.70	1.27-3.83	9.8-28.7
6	7.07-11.10	5.37- 9.12	1.39-2.54	14.8-27.5
7	6.70- 9.92	4.71- 8.25	1.58-2.76	16.9-31.8

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[4] Zimmermann, K. W., *Arch. mikr. Anat.*, 1911, **78**, 199.

[5] Peter, K., Untersuchungen über Bau und Entwickelung der Niere, Jena, 1909.

[6] Modell, W., *Anat. Rec.*, 1933, **57**, 13.

[7] Nakamura, S., *Fol. Anat. Jap.*, 1935, **13**, 45.

The average percentage length of the second segment in the 4 animals is 17.7, 15.5, 20.7 and 23.9% respectively. The average for the entire series of 60 tubules is 19.5%.

The evidence for the subdivision of the mammalian proximal convoluted tubule into at least 2 cytologically distinct segments is entirely convincing. A more extensive segmental differentiation has been postulated by Suzuki,[8] but no critical cytological proof in support of his contention has yet been adduced.

8557 C

Effect of Combined Administration of Oestrone and Progesterone in Adult Ovariectomized Rats.

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Previous experiments[1, 2, 3, 4] have shown that the mucification of the vagina which is usually observed in pregnant rats may be elicited during lactation if large doses of oestrin are administered. Since administration of similar doses of oestrin in ovariectomized rats would lead to cornification of the vaginal epithelium, the mucification reaction was ascribed to the simultaneous action of oestrin and corpus luteum hormone. Similarly the epithelial lining of the uterus shows only a moderate degree of progestational proliferation during lactation or pseudo-pregnancy in the rat, while in the second half of pregnancy the proliferation of the endometrium and the growth of the uterus *in toto* is much more marked and of a different nature. Various reasons which are discussed in more detail in the papers quoted above led us to the conclusion that the endometrial changes during lactation, pseudo-pregnancy, and early pregnancy, are mainly due to the hormone of the corpus luteum, and we called this reaction "the first stage of progestational proliferation." The "second stage of progestational proliferation" is that observed during late pregnancy, which we believed to be caused by the simultaneous action of corpus luteum hormone and oestrin. We concluded from indirect evidence that oestrin is produced in large quantities during late pregnancy in the rat but not during lactation or early pregnancy. This would explain why vaginal mucification and the second stage of progestational proliferation appears in late pregnancy only and not during early pregnancy or lactation.

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[8] Suzuki, T., Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen, Jena, 1912.

[1] Selye, H., Harlow, C., and McKeown, T., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1253.

[2] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinol.*, 1935, **19**, 151.

[3] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1377.

[4] Selye, H., and McKeown, T., *Proc. Roy. Soc.*, B, 1935, **119**, 1.

Meyer and Allen[5] stated that vaginal mucification may be obtained in the ovariectomized mouse by the simultaneous administration of oestrone and a corpus luteum extract. But this corpus luteum extract contained oestrin and gave either mucification or cornification by itself, depending on the dose given. Similar results have been obtained by Desclin[6] in non-castrate guinea pigs with a corpus luteum extract which also caused mucification by itself. Fels,[7] however, found that purified corpus luteum hormone preparations have no effect on the vagina of the rat, while small doses of oestrin will cause mucification, as had already been established by previous investigators. He concludes that mucification is an oestrin reaction. He states, furthermore, that although oestrin may inhibit the action of corpus luteum hormone, corpus luteum hormone cannot inhibit the effect of oestrin. He considers the experiment of Fremery, *et al.*,[8] who inhibited the vaginal effect of slightly more than one mouse-unit of oestrin with 3 rabbit-units of corpus luteum hormone in ovariectomized rats, as insignificant because of the large quantity of corpus luteum extract which had to be injected, and might have had a non-specific oestrus-inhibiting effect. Brouha and Simonnet,[9] on the other hand, state that corpus luteum extracts inhibit the oestrus cycles in normal females but do not inhibit the vaginal effect of oestrin in the ovariectomized rat; from which they conclude that corpus luteum hormone acts through the ovary. In an endeavor to obtain further evidence concerning the factors responsible for the changes occurring in the uterus and the vagina of the rodent during late pregnancy we performed a series of experiments on 6 adult ovariectomized rats which received 30 γ of oestrone in 0.5 cc. and 400 γ of progesterone in 0.4 cc. of oil daily by means of subcutaneous injections. The progesterone was prepared from pregnandiol by the method of Butenandt and Schmidt.[10] During the experiment the vaginal smear remained dioestric, except in one case in which cornified cells predominated. Histological examination after autopsy on the 20th day of treatment showed that the vagina was lined with a very high mucified epithelium in all cases and the uterus was in the second stage of progestational proliferation in all but 2 cases. These last 2 showed the picture of full oestrus, although the vagina was mucified. It seems from these experiments that progesterone changes the vaginal effect of oestrin and causes mucification. The mucification thus obtained was extremely marked and should not be confused with the slight mucin secretion which may be obtained by sub-threshold doses of oestrin in the castrate rodent. In the latter case, the mucification and the development of the epithelium will always be poor and cannot be improved by raising the dose, since this would lead to cornification. We may conclude, therefore, that both the uterine and the vaginal changes of the late-pregnant rodent are the result of the combined action of oestrin and corpus luteum hormone. It is of interest to mention in this connection that the mammary glands of our animals showed only a very slight development—hardly more than that which the oestrone in itself would have caused.

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[5] Meyer, R. K., and Allen, W. M., *Anat. Rec.*, 1933, **56**, 321.

[6] Desclin, L., *Compt. rend. Soc. Biol.*, 1933, **115**, 439.

[7] Fels, E., *Arch. f. Gynäkol.*, 1934, **158**, 364.

[8] Fremery, P. de, Luchs, A., and Tausk, M., *Pflüger's Arch.*, 1932, **231**, 341.

[9] Brouha, L., and Simonnet, H., *Ann. de Physiol.*, 1929, **5**, 567.

Summary. The effect of 30 γ of oestrone per day on the vagina and uterus of the ovariectomized rat is modified by the simultaneous administration of 400 γ of synthetic progesterone. The vaginal epithelium shows mucification and the uterus is in the second stage of progestational proliferation. The vaginal effect of oestrone may be modified even in cases in which the uterine effect is not changed.

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[10] Butenandt, A., and Schmidt, J., *Ber. deutsch. chem. Ges.*, 1934, **67**, 1901.

8558 C

Effect of Testosterone on the Mammary Gland.

H. SELYE, C. S. MCEUEN AND J. B. COLLIP.

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The fact that derivatives of the male hormone may exert an action on the female sex organs is known from the work of Butenandt and Hanisch,[1] who showed that androstenedione which is inactive in castrate females produces vaginal oestrus in the presence of an ovary, and from the work of Parkes,[2] who found that the same is true in the case of androstanediol. The action of these compounds might possibly be explained by a transformation of these substances into oestrin by the ovary. This would seem all the more likely since Collip, Browne and Thomson[3, 4, 5] have shown that the ether-insoluble fraction of oestrin extracted from pregnancy urine (Emmenin) is relatively inactive in the spayed female, while it is much more active in the presence of an ovary. Several compounds of the androsterone-testosterone series, however, do also cause vaginal cornification in ovariectomized female rats, as shown recently by Deanesly and Parkes[6] and Korenchevsky and coworkers,[7] and it is still to be seen whether this is due to a transformation of these compounds in the female organism into female hormones by tissues other than the ovary, or whether male hormone may stimulate the genital organs of the female directly. It seems of interest in this connection that we were able recently to obtain milk secretion, that is the development of a female characteristic even in the male, both normal and castrate, by the administration of testosterone, the most active compound of the male hormone series, first isolated by Laqueur, *et al.*[8] from testes. The same hormone also causes milk secretion in normal and ovariectomized females. In all our experiments we used crystalline synthetic testosterone-benzoate.*

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[1] Butenandt, A., and Hanisch, G., *Ber. d. chem. Ges.*, 1935, **68**, 1859.

[2] Parkes, A. S., *Chem. and Industry*, 1935, **54**, 928.

[3] Collip, J. B., *Proc. Calif. Acad. Med.*, 1930, **1**, 38.

[4] Browne, J. S. L., *Can. J. Res.*, 1933, **8**, 180.

[5] Collip, J. B., Browne, J. S. L., and Thomson, D. L., *Endocrinol.*, 1934, **18**, 71.

[6] Deanesly, R., and Parkes, A. S., *Brit. Med. J.*, 1936, No. 3918, 257.

[7] Korenchevsky, V., Dennison, M., and Simpson, S. L., *Biochem. J.*, 1935, **29**, 2534.

[8] David, K., Dingemanse, E., Freud, J., and Laqueur, E., *Hoppe-Seylers Ztschr.*, 1935, **238**, 281.

* We are greatly indebted to Prof. Schoeller of the Schering-Kahlbaum Co. for the testosterone used in these experiments.

Four female rats 23 days of age were injected with 200 γ of testosterone-benzoate in corn oil daily subcutaneously for 23 days. At the end of this period their mammary glands showed slight development of acinar tissue and the ducts of the gland were filled with dense milk. Two non-injected controls showed only undeveloped ducts without secretion. The same degree of development was also observed in 4 ovariectomized females treated for 23 days with the same amount of testosterone. The mammary glands of 2 ovariectomized, non-injected animals proved to be negative. Three 25-day-old males, injected with the same daily dose of testosterone

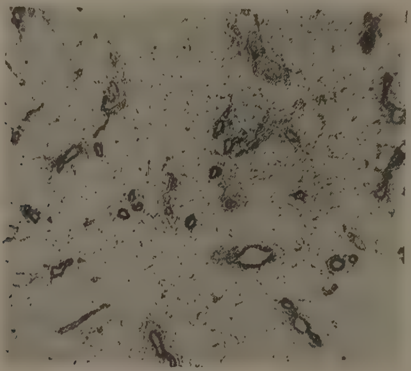

Fig. 1.
Mammary gland of a castrate male control, not injected.

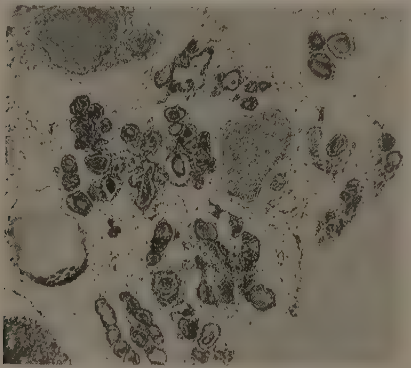

Fig. 2.
Mammary gland of a male injected with 200 γ of testosterone-benzoate for 23 days.

for 14 days, and 3 others injected for 22 days also showed slight development of the mammary gland and more or less abundant milk secretion. The same development and secretion was also obtained in 3 castrate males injected for 14 days, and 3 castrate males injected for 23 days with 200 γ of testosterone-benzoate per day (Figs. 1, 2, 3). It may be of interest to recall that a similar change in the mammary gland, that is a slight degree of development with marked milk secretion, was observed in rats ovariectomized during gestation. In these rats the vaginal epithelium was not mucified nor cornified and the formation by the placenta of a special mammary gland influencing hormone, other than oestrin, had to be assumed.[9] It remains to be seen whether the milk secretion inducing effect of the male hormone is due to a direct action on the mammary gland or to a stimulation of the hypophysis.

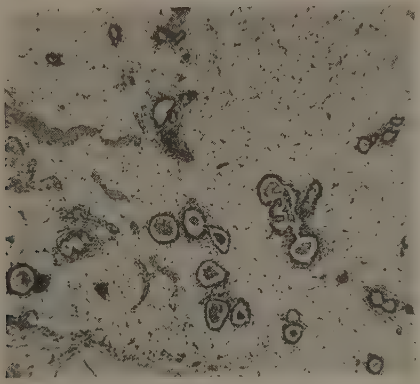

FIG. 3.
Mammary gland of a castrate male injected with testosterone-benzoate for 14 days.

Summary. Two hundred γ of synthetic testosterone per day will cause slight development of mammary tissue and marked milk secretion in immature males and females, both in the presence and in the absence of the gonad.

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[9] Selye, H., and McKeown, T., *Proc. Roy. Soc.*, B, 1935, **119**, 1.

8559 C

Mammary Gland Development in the Hypophysectomized Albino Rat.*

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Numerous investigations on mammary gland development in recent years have indicated that the ovarian hormones, estrone and progestin, are responsible for the growth of the glands. However, since increasing knowledge of the physiology of the pituitary gland seems to give it a central position in the endocrine system, investigators have become interested in determining whether or not the mammary glands could be experimentally developed in hypophysectomized animals.

Pencharz and Long[1] who hypophysectomized rats in late pregnancy (20th and 21st days) concluded that mammary development continued in the absence of the pituitary. However, at this stage of pregnancy growth is complete and lactation is being initiated. In fact, Selye, Collip and Thomson[2] observed that milk secretion always set in normally after parturition, but stops after a few hours, in rats hypophysectomized during the latter half of pregnancy. The mammary glands of rats hypophysectomized during the second half of pregnancy have been studied cytologically by Jeffers.[3] The epithelial cells were observed to resemble those of glands from normal pregnant animals. These studies appear to indicate that the development of the mammary gland which normally occurs during the second half of pregnancy can continue in the absence of the pituitary. However, lactation fails within a few hours after parturition. Selye, Collip and Thomson[4] found that the continued application of large doses of estrone in lactating rats failed to lead to repair of the rapid involution of the mammary gland which takes place after hypophysectomy. They were of the opinion that the presence of the pituitary gland is necessary for the action of estrone upon the mammary glands.

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† Department of Anatomy, Yale University Medical School, Visiting Research Assistant Professor of Dairy Husbandry during summer of 1935.

‡ Aided in part by a grant of the Committee on Grant-in-Aid of the National Research Council.

[1] Pencharz, R. I., and Long, J. A., *Am. J. Anat.*, 1933, **53**, 117.

[2] Selye, H., Collip, J. B., and Thomson, D. L., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 589.

[3] Jeffers, K. R., *Am. J. Anat.*, 1935, **56**, 279.

[4] Selye, H., Collip, J. B., and Thomson, D. L., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1377.

Although the above studies indicate that the hypophysis plays no part in the development of the mammary glands during the second half of pregnancy, and that it may play some rôle in preventing the rapid involution of lactating glands, they bring us no closer to an understanding of the part played by the pituitary gland, if any in the growth of the mammary gland. Ruinen,[5] in injecting hypophysectomized male rats (90 to 120 gm. in weight) subcutaneously, twice daily for 14 days after operation, with 50 mouse units of crystalline menformon (keto-hydroxy-oestrin) in 0.1 cm. of oil, secured mammary development in 4 of these which was comparable with that which one usually sees in animals under the same menformon treatment with their hypophysis intact. Freud and De Jongh[6] treated a series of young castrated female rats the first two weeks with 100 International units of menformon per day. The animals were then hypophysectomized and during a following week they were treated with menformon and progestin. These investigators observed an extension of the alveolar system with side branchings along the ducts and concluded that the hypophysis of rats is not involved in the changes of the mammary glands produced by progestin. Asdell and Seidenstein[7] ovariectomized a series of rabbits and then kept them for 2 months to exhaust their supply of ovarian hormone. The animals were then hypophysectomized and the injections begun 2 months later. The rabbits were injected daily with 25 R. U. of progynon-B and 0.5 cc. of progestin (equal to about 4 rabbit units). The animals were injected for 15 days and then sacrificed. They state that the degree of development of the mammary glands was about the same in hypophysectomized animals as in those in which the hypophysis was not disturbed or was incompletely removed, and conclude that mammary gland development can occur in the absence of the hypophysis. Nelson[8] reported mammary development in 4 male hypophysectomized guinea pigs, treated with 40 R. U. of oestrone daily, comparable to that observed in normal guinea pigs.[9] Lyons and Pencharz[10] secured nipple development in 4 hypophysectomized male guinea pigs which had been injected for 2 months with progynon-B, almost equal to that which they obtained in 6 normal male animals under similar treatment. However, there was only a limited growth of ducts and alveolar buds in the hypophysectomized animals in comparison to the functional alveolar development found in the injected intact males.

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[5] Ruinen, F. H., *Acta Brevia Neerl.*, 1932, **2**, 161.

[6] Freud, J., and De Jongh, S. E., *Acta Brevia Neerl.*, 1935, **5**, 47.

[7] Asdell, S. A., and Seidenstein, H. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 931.

[8] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 222.

In order to further investigate whether or not the pituitary gland plays any rôle in bringing about the growth of the mammary gland when animals are under the influence of estrogenic substances, we have hypophysectomized a series of immature albino rats and subsequently treated them with varying amounts, 25 to 500 International units per day, of the benzoate of hydroxyestrin (Progynon-B§). Of the animals which were operated upon there were 5 females and 4 males which survived 15 days or longer and in which the pituitary gland had been completely removed as was revealed at autopsy. The results are summarized in Table I.

TABLE I.

Rat No.	Sex	Date of Birth	Date of Castration	Date of hypophysectomy	Date injections began	Dosage in I.U. per day	Days injected	Evidence of mammary gland growth
3	F	5-15	6-14	7-3	7-15	25	35	No
12	F	5-16	7-5	7-9	7-15	75	45	No
17	M	5-16	—	7-5	7-7	25	26	No
19	M	5-22	—	7-2	7-6	75	20	No
48	F	6-29	—	8-7	8-9	250	15	No
49	M	6-29	—	8-5	8-9	500	15	No
50	M	6-29	—	8-7	8-9	100	18	No
51	F	6-29	—	8-7	8-9	100	23	No
52	F	6-29	—	8-5	8-9	500	24	No

None of the mammary glands from these 9 hypophysectomized immature rats showed any evidence of growth. The duct system was limited and showed only few end-buds. In comparison with these mammary glands, the glands from control animals which had been castrated, subjected to similar treatment, but whose pituitary glands were intact and in animals whose pituitary glands were incompletely removed developed an extensive duct system with numerous end-buds. Even the mammary glands of 3 females whose ovaries were intact and in which injections were begun 2 to 4 days after hypophysectomy showed no mammary gland growth.

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[9] Nelson, W. O., and Smelser, G. K., *Am. J. Physiol.*, 1933, **103**, 374.

[10] Lyons, W. R., and Pencharz, R. I., Proc. Soc. Exp. Biol. and Med., 1936, **33**, 589.

§ The Progynon-B used in this study was kindly supplied by Dr. E. Schwenk of the Schering Corporation.

Conclusion: Progynon-B injected subcutaneously into 5 female and 4 male, immature, hypophysectomized albino rats in dosages varying from 25 to 500 International Units per day, produced no evidence of mammary gland growth.

8560 P

Action of Oestrin on the Vagina During Lactation.

Marius Votquenne.* (Introduced by E. C. Cutler.)

From the Laboratory of Surgical Research, Harvard Medical School, Boston, Massachusetts.

The injection of oestrin during lactation has resulted in contradictory opinions regarding the vaginal reaction. Previous experiments on the ovarian hormonal antagonism[1] have shown that the injection of 18, 10, 6, 4, and 2 rat units of oestrin, in oily solution, daily during the first 5 days of lactation always provoked typical oestrus as indicated by vaginal smears. Doses of less than one rat unit have demonstrated the existence of different thresholds of anti-luteinic action of oestrin, the vaginal reaction of oestrus being the first to appear.

Recently, Selye and co-workers have observed that doses of thirty micrograms of Oestrone, in oily solution, daily from the fifth to the tenth day of lactation have failed to provoke vaginal oestrus.[2, 3] They also reported that 100 or 500 micrograms of Oestrone, injected daily from the fourth to the fourteenth day, had no influence on the vaginal smear which remained typical of dioestrus. "The vaginal epithelium showed a high degree of mucification, a condition regularly seen in pregnancy but not in lactation." It seems difficult to accept this hypothesis of complete antagonism of corpora lutea to the vaginal reaction due to oestrin since a decidual reaction is not maintained under conditions of follicular dominance.

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* C. R. B. Educational Foundation Fellow.

[1] Votquenne, M., *Compt. Rend. des Seances de la Soc. de Biol.*, 1934, **117**, 1121.

[2] Selye, H., Harlow, C., and McKeown, T., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1253.

[3] Selye, H., Collip, J. B., and Thomson, D. L., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1377.

Further experiments with the injection of oestrin during lactation confirm earlier work.[1] The typical vaginal histology of oestrus has been obtained with 0.2 rat unit of oestrin injected daily during the first 4 days of lactation, and the animal sacrificed on the fifth day, even though the vaginal smear remained falsely negative. Particular attention must be paid to the fact that the vaginal smear during lactation does not correspond to the true state of the vaginal epithelium since complete rut is not attained with doses of oestrin of less than one rat unit (Allen and Doisy). Even if the vaginal epithelium is keratinized during lactation marked leucopedesis may occur and the smears show either typical dioestrus or a mixture of leucocytes and cells at all possible stages of keratinization, or even complete desquamation in casts. Vaginal keratinization seems to be the primary effect of oestrin during lactation and the leucocytes disappear only after it is complete.

Injections made during the first 5 days of lactation act when corpora lutea are progressively developing their power. However, similar results have been obtained by injecting oestrin ("Theelin in oil"—Parke, Davis & Co.) daily from the fifth to the ninth day, i. e. when the corpora lutea are fully developed (Criterium: Loeb's deciduoma). The observations of Selye and his co-workers can not be duplicated using smaller, more physiological doses of oestrin. Histological examination of the vaginal wall has shown that the typical picture of oestrus is obtained by the daily injection of 0.75 to 1 rat unit of theelin in oil from the fifth to the ninth day of lactation.

It seems difficult to defend the hypothesis of the anti-oestral action of corpora lutea during lactation under these circumstances. The injection of theelin during lactation may induce the typical histological reaction of oestrus in the vaginal epithelium without characteristic changes in the smear. The oestrin and progestin may act simultaneously either on different organs (vaginal oestrus and deciduoma) or on the same organ (leucocytes and keratinized cells at all stages). These results confirm the interrelationship of the differential and reciprocal thresholds of action between oestrin and progestin.

Summary. The anti-oestral action of corpora lutea of lactation is observed only when doses of less than one rat unit are given. The vaginal mucification observed by Selye and his co-workers is probably due to the large doses of Oestrone injected, rather than being the expression of a physiological anti-oestral action of the corpora lutea.

8561 C

Nature of the Antibodies for Sheep-Cells in Infectious Mononucleosis.

C. A. Stuart, A. M. Griffin, MacDonald Fulton and E. G. E. Anderson.

From Brown University and Charles V. Chapin Hospital, Providence, R. I.

The sheep-cell antibodies in infectious mononucleosis serums have been called heterophile or Forssman antibodies by several investigators but since, as previously pointed out,[1, 2, 3, 4] the Forssman character of these antibodies is questionable, further studies on their nature are necessary. To this end the relationship of alcoholic extracts of beef- and sheep-cells to the sheep-cell antibodies in infectious mononucleosis and in several Forssman heterophile serums has been determined.

Extracts were prepared by treating one volume of packed cells with 5 volumes of alcohol for 12 hours at room-temperature followed by 8 hours in a mechanical shaker, filtering, and concentrating the filtrate to one-half its volume at 37°C. Equal volumes of this extract and saline were mixed and the alcohol removed by evaporation at 50°C. The turbid suspensions prepared in this manner were used to inhibit the hemolysis of sheep-cells by the various normal and immune serums noted in Table I.

In the inhibition-tests 0.25 ml. of the alcoholic extract in dilutions ranging from 1:1.25 to 1:320 was placed in a series of tubes; 0.25 ml. of the normal or immune serum containing sufficient hemolysin to hemolyse completely the dose of sheep-cells in 10 minutes, and 0.25 ml. of guinea pig-complement diluted 1:20 was added to each tube in the series. Triplicate controls with 0.25 ml. of saline substituted for the extract were used in each test. After 15 minutes' incubation at 37°C. in a water-bath, 0.25 ml. of a 1.0% sheep-cell suspension was added to each tube. When all 3 control tubes showed complete hemolysis the degree of hemolysis in the tubes containing the extract was noted. Ten minutes later a second reading was taken. The anticomplementary action of the extracts themselves was controlled by using pure isophile immune sheep-serum (prepared by absorbing rabbit antisheep-cell serum

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[1] Stuart, Burgess, Lawson and Wellman, *Arch. Int. Med.*, 1935, **54**, 199.

[2] Bailey and Raffel, *J. Clin. Invest.*, 1935, **14**, 228.

[3] Stuart, Proc. Soc. Exp. Biol. and Med., 1935, **32**, 861.

[4] Davidson and Walker, *Am. J. Clin. Path.*, 1935, **5**, 455.

TABLE I.

Inhibition of Sheep Lysins in Various Serums by Alcoholic Extracts of Sheep- and Beef-Cells.

Serums tested	Number tested	Dilutions of Sheep extract 5	20	80	320	1280	Beef extract 5	10	20
Rabbit antisheep	2	C	C	C	P	—	—	—	—
,, antiguinea pig-kidney	2	C	C	C	P	—	—	—	—
,, antihuman A	2	C	C	C	P	—	—	—	—
*Normal human	6	C	C	C	P	—	—	—	—
Serum-sickness	5	C	C	C	P	P	—	—	—
Infectious mononucleosis	8	P	—	—	—	—	—	—	—
Pure isophile sheep	1	—	—	—	—	—	—	—	—

C = complete inhibition. P = partial inhibition. — = no inhibition.
*Selected for high sheep-cell lysin-titers.

TABLE II.

Adsorption of Sheep-Cell Antibodies from Infectious Mononucleosis Serum (55) with Alcohol-Extracted Sheep- and Beef-Cells.

	Sheep-agglutinins 20	40	80	160	320	640	1280	2560	Sheep-lysins 20	40	80	160	320	640	1280	2560	5120
Adsorbed with alcohol-extracted sheep-cells																	
Titer before adsorption	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	2
,, after first adsorption	4	4	4	4	4	2	—	—	4	4	4	4	4	3	1	—	—
,, ,, second ,,	4	4	4	4	2	—	—	—	4	4	4	3	1	—	—	—	—
,, ,, third ,,	4	4	2	—	—	—	—	—	4	3	1	—	—	—	—	—	—
,, ,, fourth ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Adsorbed with alcohol-extracted beef-cells																	
Titer after first adsorption	4	2	—	—	—	—	—	—	4	4	3	—	—	—	—	—	—
,, ,, second ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

with boiled sheep- and raw beef-cells) as the hemolysing agent. In Table I is shown the inhibitory action of the alcoholic extracts of beef- and sheep-cells on the various serums. Though the second reading, taken 10 minutes after the controls were completely hemolysed, showed slightly less inhibitory action on the part of the extract it is a more rigid test and only this reading is recorded in the table.

It will be seen that the alcoholic extract of sheep-cells possessed marked inhibitory action on the 5 kinds of serums containing known heterophile lysins for sheep-cells, i. e., rabbit antisheep-cell, rabbit antiguinea pig-kidney, rabbit antihuman A, normal human and serum-sickness serums. Alcoholic extracts of beef-cells on the other hand showed no inhibitory action on any of the serums. Neither the beef- nor sheep-cell extracts inhibited the sheep-cell lysins of infectious mononucleosis. The partial inhibition in the infectious mononucleosis serums in the 1:5 dilution of the sheep-extract is doubtless accounted for by the presence of normal heterophile antibodies in these serums. Obviously, then, that fraction of the beef- and sheep-cell which adsorbs the sheep-cell antibodies of infectious mononucleosis is not alcohol-soluble, in contradistinction to the Forssman heterophile antigen.

The relationship of the sheep-cell antibodies in infectious mononucleosis to "alcohol-precipitated" and "alcohol-extracted" sheep- and beef-cells was also determined. The former were prepared by adding the cells to alcohol. When coagulation was complete the alcohol was immediately decanted and the cells dried in a current of warm air. Cells were extracted with 3 changes of alcohol for 7 days with frequent shaking. The alcohol was decanted and the residue after drying at 37°C. constituted the extracted cells.

Neither the alcohol-precipitated nor the alcohol-extracted sheep- or beef-cells adsorbed any appreciable amount of either agglutinins or lysins from isophile sheep-serum. Both the alcohol-precipitated and extracted sheep- and beef-cells, however, completely removed agglutinins and lysins for sheep-cells from infectious mononucleosis serums. Due, doubtless, to the physical characteristics of alcohol-treated cells the antibodies were adsorbed more slowly than by the equivalent raw or boiled cells. Table II shows the adsorption of the serum of case 55 with alcohol-extracted cells. The serum was adsorbed 4 times for one hour each at 37°C. and tested after each adsorption. It will be noted that while 4 adsorptions with the extracted sheep-cells were required to remove completely the sheep-cell antibodies, extracted beef-cells removed them in 2 adsorptions. This difference in the action of sheep- and beef-cells on such antibodies has been noted before with raw and boiled cells[1] and it would seem that beef-cells possessed per unit-volume a greater amount of the antigen for the sheep-cell antibodies of infectious mononucleosis than did sheep-cells themselves.

The results of the inhibitory and adsorptive experiments agree in showing that the sheep-cell antibodies in infectious mononucleosis are not Forssman antibodies, for they react with alcohol-treated sheep- and beef-cells but not with the alcoholic extracts of these cells.

8562 C

A Thermostable Antigen in Beef-Cells.

C. A. STUART, A. M. GRIFFIN, K. M. WHEELER AND
SHIRLEY BATTEY.

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Bailey and Raffel[1] showed that serums from 3 patients with infectious mononucleosis contained lysins for beef-cells in addition to agglutinins and lysins for sheep-cells.* Serums from 22 cases of infectious mononucleosis examined by us showed the presence of beef-cell lysins in dilutions from 1:1280 to 1:20480, much higher than the titer for sheep-cell lysins. Beef-cell agglutinins were not present in any of the 22 serums in a concentration exceeding that in normal serum. In this respect the antigen responsible for the beef-cell antibodies in infectious mononucleosis acts like a true Forssman antigen in that high lytic titers are produced with little or no corresponding agglutinins.

It has been shown that both raw and boiled beef- and sheep-cells adsorb the beef-lysins of infectious mononucleosis[1] and we have amply confirmed this fact. The presence of a thermostable antigen in the beef-cell, in which no heterophile antigen has yet been demonstrated, needs further investigation. Accordingly the relationship of the beef-cell lysins of infectious mononucleosis serum to the alcoholic extracts and alcohol-treated sheep- and beef-cells previously described[2] was determined. The anticomplementary action of the extracts was controlled with pure isophile beef-serum prepared by adsorbing rabbit antibeef-cell serum with raw sheep-cells. The possibility of hemolysis by the complement must not be overlooked in testing for beef lysins. The complement from 25 of 43 guinea pigs had to be discarded because of the presence of normal beef-lysins in dilutions of 1:10 to 1:100. Sheep-lysins were also present in 1:40 dilution in the serum of 2 animals. These antibodies were not adsorbed by boiled beef-cells, which indicates their isophile nature.

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[1] Bailey and Raffel, *J. Clin. Invest.*, 1935, **14**, 228.

* Agglutinins and lysins for horse cells are also present in infectious mononucleosis. In a group of 15 serums the titer of these antibodies ranged from 1:1280 to 1:40960.

Alcoholic extracts of sheep- and beef-cells failed to inhibit the beef-lysins of infectious mononucleosis serums. Both the alcohol-precipitated and extracted sheep- and beef-cells, however, adsorbed the beef-lysins from such serums; the alcohol-treated sheep-cells were much less efficient in this respect. Beef-cells extracted with alcohol in a Soxhlet apparatus for 24 hours retained their ability to adsorb the beef-lysins. Neither the alcohol-precipitated nor extracted cells adsorbed any of the antibodies from the isophile beef-serum.

From the present and previous investigations[2] it is obvious that the antigen of the beef-cell and sheep-cell which adsorbs the antibodies of infectious mononucleosis is not the well-known isophile fraction common to these cells since it is thermostable and "alcohol-resistant." Nor is it a Forssman heterophile antigen since it is not alcohol-soluble. This antigen like the Forssman antigen appears to have widespread distribution since it has been found in horse tissue,[1, 3] *Clostridium welchii*[1] and, as we shall show presently, in the rabbit. For convenience this antigen will be termed the B. T. (beef-thermostable) antigen.

While this antigen is not present in rabbit cells[4] nor rabbit-tissue[3] the immunization of rabbits with raw beef-cells produces no antibodies that can be removed by boiled beef-cells.[5] These facts led us to investigate the serum of the rabbit for the presence of the B. T. antigen. The serums from 6 normal rabbits were used to inhibit the beef-lysins in 5 infectious mononucleosis serums. Table 1 shows that the rabbit-serums markedly inhibited the beef-lysins. Similar experiments with 8 infectious mononucleosis serums

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[2] Stuart, Griffin, Fulton and Anderson. In press.

[3] Davidsohn and Walker, *Am. J. Clin. Path.*, 1935, **5**, 455.

[4] Stuart, Tallman and Brintzenhoff, *J. Immunol.*, 1935, **28**, 85.

[5] Hyde, *Am. J. Hyg.*, 1925, **5**, 217.

TABLE I.

Inhibition of Beef-Lysins in Infectious Mononucleosis Serums by Normal Rabbit- and Horse-Serums.

Normal Rabbit-Serums*	Serum-dilutions					
	20	40	80	160	320	640
4382	C	C	C	P	P	—
4638	C	C	P	P	—	—
4204	C	C	C	P	P	—
4414	C	C	P	P	—	—
4381	C	C	P	P	—	—
4248	C	C	P	P	—	—
‡Iso. beef-serum	—	—	—	—	—	—

Normal Horse-Serum†	Serum-dilutions				
	5	10	20	40	80
1471	C	P	P	—	—
1472	C	P	P	—	—
1888	C	C	P	P	—
1892	C	C	P	P	—
‡Iso. beef serum	—	—	—	—	—

*Each rabbit-serum tested with 5 infectious mononucleosis serums.
†Each horse-serum tested with 8 infectious mononucleosis serums.
‡All rabbit- and horse-serums negative in anticomplementary control.
C = complete, P = partial inhibition.

demonstrated the presence of the B. T. antigen in 4 horse serums though in lower concentration.

The presence of the B. T. antigen in horse-serum is particularly important because it may account for the antibodies for beef- and other cells that are often found in the serums of humans treated with horse-serum. Serums from 5 cases of horse-serum sickness containing beef-agglutinins and lysins as well as sheep-agglutinins were adsorbed with boiled beef-cells. The agglutinins and lysins for beef- and agglutinins for sheep-cells were completely removed by a single adsorption. Despite the fact that the boiled beef- and sheep-cells completely adsorb the sheep-agglutinins from both infectious mononucleosis and serum-sickness, these agglutinins in the 2 serums are not identical since boiled rabbit-cells will adsorb the sheep-agglutinins of serum-sickness but not infectious mononucleosis.[6] There must consequently be a second thermostable antigen in the beef-cell which is present also in the rabbit-cell, the sheep-cell and horse-serum. This second antigen is probably related to but cannot be identical with the B. T. antigen.

A thorough investigation of these thermostable antigens can only be made when animals are found which lack these antigens and therefore possess the ability to produce antibodies. Man appears to lack them because antibodies of the type which they would produce are found in cases of infectious mononucleosis and serum-sickness. Moreover in 3 normal human serums containing beef-lysins in low concentration these antibodies were completely adsorbed by boiled beef-cells. It might seem from the work of Bailey and Raffel[1] that animals lacking the B. T. antigen were rather common. But it should be pointed out that a test of the erythrocytes, the tissue or the serum alone is quite inadequate for the proper classification of an animal with respect to this antigen. Table II shows the presence and location of the B. T. antigen in those animals that have been studied most extensively. The absence of the B. T. antigen in the guinea pig strongly suggests that the sheep- and beef-cell antibodies produced in these animals by immunization with boiled sheep-cells[7] were actually antibodies from the B. T. antigen in these cells.

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[6] Stuart, Fulton, Ash and Gregory. In press.

TABLE II.
Distribution of the B. T. (beef-thermostable) Antigen in Certain Animals.

	Erythrocyte	Serum	Tissue
Beef	+	+	—
Sheep	+	+	—
Horse	*+	+	+
Rabbit	—	+	—
Guinea pig	—	—	—
Human	—	—	

*Horse-cells did not completely adsorb the beef-lysins from infectious mononucleosis serums. A single adsorption reduced the beef-lysin titer about 90%, but no further decrease occurred on subsequent adsorptions.

The experiments herein described indicated the existence of a class of antigens which are thermostable, insoluble in alcohol, but not activated by exposure to that reagent. The properties of the type-specific antigens, K_3 and K_5, found in rabbits[8] would seem to place them in this class. The properties of these thermostable antigens are partly those of the Forssman antigens and partly those of the isophile antigens.

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[7] Weil, *Bioch. Z.*, 1914, **58**, 257.

[8] Fischer, *J. Immunol.*, 1935, **86**, 97.

8563 C

Association of Characters Among Dissociates from *Staphylococcus aureus.**

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Pinner and Voldrich[1] found that *albus* "dissociates" of *Staphylococcus aureus* are of low virulence and produce little or no hemolysin, coagulase or gelatin-liquefying enzyme. Burnet,[2] however, reports that in the absence of CO_2 his *albus* dissociates produced more hemolysin though less coagulase than his undissociated cultures.

We have studied enzyme-production in 36 paired smooth dissociates from 15 *aureus* strains (rough variants not studied). Thirteen pairs were obtained by plating 13 stock cultures and selecting the lightest and darkest colony on each plate. Eight pairs were isolated by the same technic from 5 *aureus* strains aged for 30 days in veal-infusion-broth. Fifteen pairs were isolated from 5-day cultures grown in oxalated Walbum's medium. Relative hemolysin, fibrinolysin, coagulase, gelatin-liquefaction and violet-reaction were determined for each pair.

To determine the association between any 2 characters, the entire population (72 dissociates) was divided as follows: *Group AB,* cultures in which relatively-high character A is associated with relatively-high character B, when comparison is made between the 2 paired dissociates from the same parent strain. *Group ab,* cultures in which relatively-non-high A (i. e. equal or relatively-low) and relatively-non-high B are similarly associated. *Groups Ab and aB,* cultures in which high A is associated with non-high B; or non-high A with high B.

The approximate ratio ("betting odds") in favor of a parallel quantitative variation in A and B was calculated by the formula, $R = (AB+ab)/(Ab+aB)$. The mathematical coefficient of association[3] was determined by the formula:

$$Q = \frac{(AB)\ (ab) - (Ab)\ (aB)}{(AB)\ (ab) + (Ab)\ (aB)}$$

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* Work supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Pinner, M., and Voldrich, M., *J. Infect. Dis.*, 1932, **50**, 185.

[2] Burnet, F. M., *J. Path. and Bact.*, 1930, **33**, 1.

[3] Yule, G. U., An introduction to the theory of statistics, 1927, p. 37.

Ratios and coefficients thus obtained are recorded in Table I.

TABLE I.

Association of Characters in *Staphylococcus aureus.*

Relative hemolysin production was determined *for each pair* by parallel tests with 48-hour peptone-ammonium lactate-broth-cultures grown in the presence of 25% CO_2. Relative fibrinolysin production was determined for each pair by the fibrin-clot technic of Tillett and Garner.[4] Relative coagulase, by the clotting time of citrated human plasma plus an equal volume of 48-hour peptone-broth-culture. Relative gelatin-liquefaction, by stab-cultures (20° C.). Relative reaction to the violet-test, by the technic of Chapman and Berens.[5]

Character A	Character B	—— Correlation-groups ——					
		AB	ab	Ab	aB	*R*	Q
Pale* color	High hemolysin	22	33	14	3	*3:1*	**89%**
" "	" gelatin-liquefaction	22	30	14	6	*5:2*	**77%**
" "	" fibrinolysin	11	36	25	0	*2:1*	**100%**
High hemolysin	" gelatin-liquefaction	21	40	4	7	*6:1*	**94%**
" "	" fibrinolysin	11	47	14	0	*4:1*	**100%**
High gelatin-liquefaction	" "	10	43	18	1	*5:2*	**92%**
Pale color	Equal* coagulase	35	1	1	35	*1:1*	**0%**
" "	" violet-reaction	35	1	1	35	*1:1*	**0%**

*In applying the formulœ relatively-pale color = A; relatively-dark color = a; equal coagulase = B; non-equal = b, etc.

[4] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[5] Chapman, G. H., and Berens, C., *J. Bact.*, 1935, **29**, 437.

The table shows that relatively-high hemolysin, relatively-high gelatin-liquefaction and relatively-high fibrinolysin are each associated with relatively-low orange-pigment (or relatively-high white-pigment) production. In contrast with this association the violet-reaction and coagulase-production are apparently constant in spite of quantitative variations in pigment-production.

8564 P

Effect of Ingestion of Acid and Alkali upon Amount of Vitamin C Found in Urine.*

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In a 3-weeks' study of the vitamin C content of the urine of normal individuals when comparable amounts of vitamin C were added to a diet free of vitamin C in the form of orange juice, cevitamic acid by mouth and cevitamic acid intravenously (Hawley, Stephens & Anderson,[1]) unexplainable variations were found in the C output of several individuals. If this method, which appears to be the most satisfactory method of determining vitamin C undernutrition, is to be of value, the variation in response must not be greater than normal biological deviation. A chance observation on a hospital patient suggested that changes in the pH of the urine might be one factor affecting the results. Marked changes in the vitamin C content of the urine in spite of constancy of intake were observed and were found to be correlated inversely with the pH. Accordingly, the experiment now in progress, and to date of 13 weeks' duration, was designed. The subjects, 3 at first and then 2, have maintained throughout a constant diet. The effect of changes in pH brought about by the ingestion of $NaHCO_3$ or of NH_4Cl on vitamin C content of the urine has been observed when the vitamin was administered as orange juice and cevitamic acid (Merck's "Cebione" supplied by them for this purpose) both by mouth and by intravenous injection.

The effect was striking and consistent. A marked decrease in output has resulted in all instances when the urinary pH was in the alkaline range of 7.5-8.1. This effect was found not to be due to a reversible oxidation. The question arises whether this decrease is due to destruction or to better utilization (increased storage) of the vitamin. One might assume the latter from the fact that foods rich in vitamin C are initially acid but basic as a result of metabolic processes. Preliminary studies on the C content of guinea pig liver (Hawley & Daggs—unpublished work) suggest that there is greater storage under the influence of the $NaHCO_3$. A

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*Work supported in part by a grant from the California Fruit Growers Exchange.

[1] Hawley, Stephens and Anderson, *J. Nutrition*, 1936, **11**, 135.

comprehensive study has been started to determine, if possible, the reason for this variation.

Summary: Alterations in urinary pH have a definite effect on the vitamin C content of the urine. The amount found is less when the reaction is in the alkaline range.

8565 P

Enhancement of Muscle Contraction after Tetanus.

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(Introduced by H. S. Liddell.)

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The contractions of sciatic-gastrocnemius preparations of *Rana pipiens* were recorded isotonically by means of a free-weighted lever loaded with 20 or 30 gm. The preparations were repeatedly stimulated with maximal break shocks at intervals of one second. At one-half minute intervals a tetanising current (10 per second) was applied for 5 seconds. The following changes in amplitude of contraction after tetanus, as compared with the amplitude before tetanus, appeared:

1. *Normal Muscle.* A. *Indirect Stimulation.* In a fatiguing muscle an increase in amplitude occurred and the percent increase became greater (up to 800%) as fatigue progressed. No change was observed in unfatigued muscle, and tetanus during this time diminished the increase to be expected in fatigue. The enhancement of contractions in a fatigued muscle was greater after tetanus (for 5 seconds) than after a rest period of similar duration, and the enhancement persisted for a greater number of stimulations.

B. *Direct Stimulation.* Fine copper wires, inserted into the belly of the gastrocnemius, were employed. No increases appeared in most of the experiments. It is concluded that in those fatigued preparations in which enhancement occurred after each tetanus some muscle fibers were being stimulated indirectly. This has been proved by the studies on curarised preparations.

2. *Curarised Muscle.* There was no enhancement after tetanus during direct stimulation of the gastrocnemius. It is therefore

concluded that the enhancement phenomenon occurs in the myoneural junction. With simultaneous tetanising of the sciatic nerve and direct stimulation of the muscle at one per second no change in the height of contractions was observed. Since in these experiments curarisation had progressed just to the point where muscle gave no response to stimulation of the motor nerve, it is assumed that autonomic nerves, if present, were not curarised. Therefore, it is highly probable that the autonomic fibers are not responsible for the enhancement phenomenon since they are much less sensitive to curare than are the motor nerves. Thus, the enhancement is not due to an Orbeli phenomenon; *i. e.,* stimulation through the sympathetic fibers in the sciatic.

3. *Lactic Acid.* Indirect stimulation of muscles treated with one percent lactic acid-Ringer's for longer than 5 minutes gave very few contractions. As the concentration or periods of immersion were reduced so that the muscle was enabled to respond to stimuli for a longer period, enhancement appeared. Such increases after tetanus were, in general, less than those exhibited by a normal muscle in about the same state of fatigue. Lactic acid apparently affects the enhancement phenomenon only by limiting the number of contractions. Therefore, lactic acid is probably not related to this phenomenon.

4. *Iodoacetic Acid.* Preparations subjected to iodoacetic acid (0.02 gm. injected into the dorsal lymph sac) and stimulated indirectly responded for only a few (30-40) contractions. Tetanus during the very brief fatigue hastened fatigue but produced no enhancement.

5. *Isometric Lever.* When the preparation was stimulated indirectly and a crude isometric lever was employed no enhancement was observed after tetanus if the initial tension on the muscle exceeded 100 gm.

The enhancement phenomenon was observed equally well in normal preparations subjected to maximal break, super-maximal make and break, and supermaximal summated stimulations.

Contracture always followed tetanus and thus caused the first 3 or 4 contractions to be reduced in amplitude as compared with those directly preceding tetanus. The following 10-15 contractions exhibited the enhancement phenomenon.

It seems probable that some mediator is quantaly produced, in excess, at the myoneural junction during tetanus and remains to increase the contractions until it is gradually dissipated. The effect of this excess is evident only in fatigued muscle. It seems unlikely that the effect is due to decreased viscosity resulting from the increased temperature which occurs in tetanus or that there is a more rapid regeneration of phosphagen during tetanus.

Work is in progress to ascertain the chemical nature of the mediator and to investigate fatigue phenomena in the single fiber preparation.

8566 C

Amino Nitrogen of Nephritic Transudates.

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It is obvious that low plasma protein is one of the factors contributing to the production of edema. Possible mechanisms responsible for the hypoproteinemia of nephritis must therefore be given careful consideration. Besides the factor of proteinuria, a leak of protein through capillary walls should seem significant. Determinations have, however, not shown a great increase in the protein content of edema fluid.

It seemed possible, though, that significant amounts of protein might be lost through capillaries and then rapidly broken down by extravascular proteolysis. This question was studied by making simultaneous determinations of amino nitrogen, by the method of Folin,[1] of serum and transudates of patients with nephritis. (These determinations were not made in the postab-

TABLE I.
Amino Acid Nitrogen, mg. % in Serum and Transudates.

Nephritic Peritoneal fluid				Nephritic Pleural fluid		Non-nephritic Transudates	
Serum	Fluid	Serum	Fluid	Serum	Fluid	Serum	Fluid
4.4	5.6	4.5	4.5	4.1	5.2	4.5	5.2
7.4	5.3	5.3	4.7	4.4	5.8	4.1	3.1
9.1	6.2	5.5	5.2	5.9	5.2	3.9	2.9
6.8	4.2	5.8	5.0	7.9	5.5	5.2	5.8
7.7	4.4	5.9	4.2	7.6	5.0	5.3	5.2
4.9	4.5	6.0	5.0			5.7	4.4
		6.2	4.6				
		Ave. 6.1	4.9	Ave. 6.0	5.3	Ave. 5.0	4.6

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[1] Danielson, Irvin G., *J. Biol. Chem.*, 1933, **101**, 505.

sorptive state and therefore the serum amino nitrogen is probably higher than the average for the 24-hour period.)

It will be seen from Table 1. that there was no evidence of rapid extravascular proteolysis.

8567 C

Glycogen Content of Freshwater Mussels During Prolonged Starvation.

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In view of the high glycogen content of the hepatopancreas of freshwater mussels,[1] the glycogen contained in the hepatopancreas and in the pedal muscle ("foot"), of 206 mussels representing 20 species, was determined during periods of starvation ranging from 2 to 536 days, to ascertain whether the utilization of stored glycogen by these invertebrates during starvation is comparable to the utilization of stored glycogen by mammals during periods of inanition.[2]

The mussels in this series were collected in September and October before the water became cold, from beds where colonies of healthy mussels were thriving, so that each animal was started in these starvation tests after a summer of normal feeding, which had prepared the animal presumably for the winter period of reduced activity. The individual mussels, between 4 and 7 years of age, were isolated either in glass hatchery jars or metal hatchery tanks, through which well-aerated water from deep wells was flowing. This water, which had been found previously to constitute a satisfactory environment for mussels as regards inorganic salts, pH, and dissolved gasses, contained no plankton, organic detritus, or organic salts. The recording thermograph showed that the water temperature fell from 18°C. in October to 11°C. in February, rising slowly to 22°C. in August and dropping to 18°C. again by October, *i.e.,* the animals under observation were subjected to a slowly changing temperature cycle comparable to that of their natural habitat.

The analyses were made by a modification of the Sahyun-Alsberg technique, using tissue frozen in carbon dioxide snow immediately

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[1] Calvin, D. B., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 96.

[2] Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

TABLE I.

% Glycogen, Dry Weight, in Hepatopancreas and in Foot of Freshwater Mussels During Starvation.

	1st month					2nd to 7th mo. incl.*					At end maximum starvation period, 5-18 mo.					
		Glycogen					Glycogen						Glycogen			
		Hepatopan.		Foot			Hepatopan.		Foot		Days of starvation		Hepatopan.		Foot	
	Cases	Max.	Aver.	Max.	Aver.	Cases	Max.	Aver.	Max.	Aver.		Cases	Max.	Aver.	Max.	Aver.
Lampsilis anodontoides	4	18.5	7.5	7.9	5.8	10	25.3	15.2	7.8	5.6	536	8	2.1	1.1	3.0	0.5
,, *carinata*	8	41.7	22.5	8.0	3.5	17	34.1	15.6	6.8	2.6	231	3	33.0	30.8	3.2	2.8
,, *recta*	—	—	—	—	—	—	—	—	—	—	189	1	43.5	43.5	4.4	4.4
,, *ventricosa*	—	—	—	—	—	—	—	—	—	—	197	1	31.1	31.1	4.9	4.9
Proptera capax	—	—	—	—	—	—	—	—	—	—	203	1	31.1	31.1	6.7	6.7
,, *purpurata*	—	—	—	—	—	—	—	—	—	—	338	1	41.4	41.4	14.5	14.5
Tritogonia verrucosa	9	27.6	11.6	8.0	4.9	17	28.5	12.6	8.2	4.6	187	5	2.0	1.9	8.2	4.9
Cyprogenia aberti	1	32.0	32.0	—	—	1	30.5	30.5	TS	TS	182	1	30.5	30.5	TS	TS
Obliquaria reflexa	1	26.1	26.1	10.0	10.0	3	22.8	13.4	8.0	6.6	163	1	6.4	6.4	8.0	8.0
Quadrula metanevra	1	28.6	28.6	7.1	7.1	4	41.6	24.3	8.9	6.8	189	2	41.6	32.3	7.6	7.0
,, *pustulosa*	4	52.2	37.8	10.6	7.2	4	18.2	7.9	10.0	3.9	161	2	1.0	0.5	TS	TS
Megalonaias gigantea	1	52.1	52.1	7.4	7.4	2	49.8	49.0	12.6	10.1	197	1	49.8	49.8	12.6	12.6
Amblema costata	5	50.8	29.1	10.2	5.9	8	23.9	11.9	10.5	6.4	238	1	TS	TS	3.7	3.7
,, *dombeyana*	3	46.0	39.1	9.2	8.0	3	44.0	27.9	5.6	4.5	189	2	44.0	36.6	5.6	5.2
Fusconaia undata	8	35.6	27.8	8.1	4.4	2	20.5	15.2	6.1	4.9	133	2	20.5	15.2	6.1	4.9
Elliptio dilatatus	1	44.6	44.6	15.2	15.2	—	—	—	—	—	231	1	TS	TS	TS	TS

*Analyses were made at intervals of 15 days or less throughout this period.

TS = too small to be measured accurately by method used.

after removal from the animal.[3] Data from 134 specimens representing 16 species have been condensed in Table 1 as typical of the findings.

From all of the analyses made on these 206 specimens it was found that, for the thick-shelled species of mussels, the maximum value of stored glycogen in terms of dry weight of the hepatopancreas lay between 25 and 61%, values between 25 and 40% being common during the first month of starvation. Although the general average glycogen value fell slowly after the first month of starvation, many individuals were found to have maintained a glycogen level above 30% during 180 to 338 days of starvation, pointing either to a slow utilization of stored glycogen, or to its replacement from within by some form of autodigestion of body tissues. However, as these mussels having high glycogen after long starvation uniformly were found to be in good condition judging by the vigorous heart action and pedal muscle activities, slow utilization of the glycogen seemed the more probable explanation for the long-sustained high level of stored glycogen.

Individuals of *L. anodontoides* were subjected to the longest period of starvation. In some specimens of this species the glycogen content of the hepatopancreas remained above the 25% level for over 200 days of starvation. After 536 days of starvation however the glycogen in the hepatopancreas averaged only 1.1%, with a maximum of 2.1% for the 8 mussels surviving this period, although the hearts of these 8 mussels were still beating normally and the pedal musculatures showed good irritability responding with weak contractions when stimulated. The actual amount of glycogen present in the hepatopancreas of 3 of these 8 mussels was too small to measure accurately by the method used, as was the glycogen in the foot of 6 of these specimens.

These specimens of *L. anodontoides* were typical of the entire starvation series when the stored glycogen in the hepatopancreas fell to 4% or lower. Although all of the species of thick-shelled mussels studied maintained in general a glycogen level in the hepatopancreas above 15 or 20% for 5 to 7 months of starvation, eventually if the starvation were continued the animals became moribund. This condition was associated with a rather sudden drop in the stored glycogen level to below 4%. This low glycogen level was attended with an oedematous condition and a discoloration of the tissues, although the heart continued to beat normally. The loss of glycogen seemed to proceed more rapidly from the hepatopancreas than from the foot of these moribund animals, so that during this stage the percent of glycogen in the foot frequently exceeded that in the hepatopancreas. *Amblema costata* and *Tritogonia verrucosa* after 238 and 187 days respectively of starvation (Table 1), are examples of this condition.

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[3] Ellis, M. M., and Calvin, D. B., *Am. J. Physiol.*, 1932, **101**, 32.

Only a limited number of thin-shelled mussels were included in these starvation tests, but in general the thin-shelled species were found less able to withstand prolonged starvation than the thick-shelled species. For example, the maximum amount of glycogen in the hepatopancreas for a group of 8 papershells (*Proptera lævissima* and *Leptodea fragilis)*, after 49 days starvation was only 6.8%, and no glycogen was found in the hepatopancreas of 3 of these individuals, and none in the foot of any of the 8.

As expected the data showed some species variation among both the thin-shelled and thick-shelled forms, but considering only the thick-shelled species it seems that the utilization of the stored glycogen by these freshwater mussels during starvation proceeds quite slowly during the first 30 to 200 days of starvation.

As may be observed, all of the glycogen values presented are based on dry weight, in an effort to avoid errors due to variation in the water content of the tissues. It was found however that variations in tissue total solids were much less pronounced even at the end of the long periods of starvation, than were the observed changes in glycogen storage, making the relative changes in glycogen even more evident.

8568 P

Action of Choline Ester on Embryonic Iris and Development and Maintenance of Reactivity.

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In a search for the mechanism of the action of choline esters, it was desired to find the earliest stage at which a choline ester produces constriction in the iris, and to follow the rate of change, if any, in the amount of constriction as the iris develops. It was also desired to find what relation this action has to the development of the muscle and of the innervation.

For the study here reported, embryo pigs were obtained at the packing house at the time of removal from the dead mother, still at approximately body temperature. Some of them were kept at body temperature, some allowed to cool at room temperature, and others were placed in Locke or other physiological solution at 0°C. Carbaminoyl choline chloride solution was placed on the cornea of the right eye and the left eye was used as a control. Sometimes the eyes were left in the head but usually they were excised and placed in glass cups. The amount of constriction produced was determined by measuring the diameter of the pupil with a pair of fine dividers and a millimeter scale and comparing it with the diameter of the control pupil.

TABLE I.
Iris (Embryo) Reactions to Carbaminoyl Choline.

Embryo		No. of eyes tested	Aver. Constr. (%)		Lysis (Notches in iris)
Length mm.	*Age da.		at 21°	at 37°	
76	52	3	0		
101	63	4	0		2-3 sml.†
103	64	10	0		?
104	64	10	0		2-4 sml.
122	71	12	3		2-7
136	76	11	6		1-6
151	81	16	13		5-6 lg.
164	87	4	20		3-4 v. lg.
177	90	5	20		
190	94	4	58		
196	95	2		0	
219	102	1	70		
’’		2		5	
220	102	2	63		
’’		1		0	
222	103	1	38		
’’		4		0	
226	104	1	78		
261	112	8	53		

*Average of values given by Lebreton and Schaeffer[6] and by Warwick.[7]
† sml., small; lg., large; v., very.

In the accompanying table some of the characteristic results are shown. It will be seen from the table (1) that at room temperature no constriction by the drug in the iris has been noted before the 71st day of gestation; (2) that there was a gradual increase in constriction from that time at least up to the 94th day; (3) that there was little or no constriction when the iris was kept at body temperature; and (4) that there was a disintegration or lysis of iris tissues at the ages from 63 to 87 days.

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[6] Lebreton, E., and Schaeffer, G., *Trav. de l'Instit. Physiol. du Fac. Méd.*, Univ. Strasb., 1923.

[7] Warwick, B. L., *J. Morph. and Physiol.*, 1928, **46**, 59.

The fact that little or no constriction was produced, by the choline ester before the 71st day, suggests that at about that time the choline-labile receptive substance was beginning to be formed. What relation this beginning of drug action has to the contact of the nerve with the muscle, we hope to determine with histologic studies now in progress.

The gradual increase in constriction from the 71st to the 94th day might by due to a gradual development of one or more of the following structures: contractile mechanism, receptive substance, nerve fiber.

The absence of constriction at body temperature suggests that the receptive substance is very labile to heat in the absence of circulating blood.

Evidence that blood may inhibit the lytic or catalytic action is found in our observation that at times the only tissue remaining after lysis is blood vascular tissue.

The lysis was often grossly observed to begin as a minute hole at the pupillary margin of the iris; *i.e.,* near the location of the sphincter muscle, on the receptive substance of which the choline ester acts. The holes developed into notches that more or less rapidly enlarged, depending on the conditions. Up to this time, lysis has been seen only in embryos from 63 to 87 days old. Since the lysis was nil or very slight in ages before constriction was produced, and was not observed in irises that might be considered completely functional, it seems that it might be due to a substance produced by the action of a choline ester on a maturing receptive substance, or on the hypothetical enzyme whose function is to build up that receptive substance. Atropine prevents the lysis and also stops it after it has begun. We are studying further the action of atropine, of physostigmine and of other drugs on this lytic process. We are also studying histologically the effects on the various tissues of this lytic action.

The finding of this lytic action seems to lend support to the hypothesis of Shaklee, Christensen and Kaplan *(l.c.)*, that the normal action of the choline ester liberated at the nerve endings is a catalytic action on the receptor substance. The hypothesis of a catalytic action appears to be in harmony, not only with the hypothesis of receptive substances by Langley,[2] so well supported by his evidence, but also with all the findings in this connection that we have examined; for example, to name only a few, the findings of Rosenblueth,[3] that there are two substances produced by the action of "sympathin" on receptive substances; the findings of Brown and Feldberg,[4] that potassium ions stimulate the curarized sympathetic ganglion which is not acted upon by acetylcholine; and the findings of Lánczos,[5] that calcium ions in addition to "sympathin" are liberated by stimulation of the sympathetic fibers to the frog's heart, and that these same calcium ions in the strength (0.01%) found in Ringer solution produced a marked action in the ergotaminized heart.

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[2] Langley, J. N., *J. Physiol.*, 1905, **33**, 374.

Further details, other drug actions and the histological studies will be presented in a following paper. We wish to thank Merck & Co. for the carbaminoyl choline.

8569 C

Bromsulphalein Dye Retention Test as a Measure of Functional Activity of Reticulo-Endothelial System.

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In the course of studies of the reticulo-endothelial system, it became apparent that some method of measurement of the function or degree of functional impairment obtained with the present so-called blockading methods, would be of great value in interpreting the experimental results. Of the various methods which offered themselves as tests, a dye excretion or clearance test, which depended upon the integrity of the reticulo-endothelial system seemed most suitable for adaptation to our needs.

Various dyes have been suggested as being selectively removed from the blood stream by the reticulo-endothelial system. Landsberger[1] reported using Congo Red for this purpose, but obtained inconstant results. Merklan and Wolf,[2] Saxl and Donath,[3] Shellong and Eisler,[4] and others have reported that phenoltetrachlorphthalein is removed from the blood stream by the reticulo-endothelial system. Herlitz[5] suggested that bromsulphalein might be removed from the blood by the macrophage system.

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[3] Rosenblueth, A., *Am. J. Physiol.*, 1932, **101**, 149; **102**, 12.

[4] Brown, G. L., and Feldberg, W., *J. Physiol.*, 1935, **86**, 10 P.

[5] Lánczos, Anna, *A. f. Path. u. Pharm.*, 1936, **180**, 312.

* Frederich Robert Zeit Fellow in Pathology.

[1] Landsberger, J., *Z. f. Immunitatsforsch.*, 1930, **65**, 385.

[2] Merklan and Wolf, *Bull. et Mem. d. l. Soc. Med. des Hosp.*, 1925, **49**, 1180.

Klein and Levinson,[6] using bromsulphalein, found that they could obtain definite retention of bromsulphalein following splenectomy, and rather more marked retention after injections of India ink in dogs. The availability of this dye, as well as its freedom from toxic manifestations led us to consider it as a measure of the functional impairment of the reticulo-endothelial system obtained with India ink and other "blockading" agents.

Bromsulphalein tests were run upon 24 normal dogs to obtain the normal values for dye clearance in the dog. Two mg. of dye per kilo body weight was injected intravenously, and blood samples drawn at 5 and 30 minutes. The blood was oxalated and centrifuged to obtain the plasma. The dye was visualized by adding 2 drops of N 10 NaOH. If the materials injected for blockade obscured the reading, precipitation with acetone was used before alkalinizing. This technique has been previously described by us.[7]

The results of the normal tests were constant for this series and no deviation in normal animals subsequently tested has been found. Values of 5% to 15% dye in the first sample and complete absence of dye in the second sample were found. No variations in dye excretion have been seen during short periods of ether or barbital anesthesia.

In the first series of experiments we were interested in confirming the observations of Klein and Levinson, that splenectomy, which may be assumed to cause little or no demonstrable liver damage and at the same time to remove a recognizable fraction of the reticulo-endothelial system, causes retention of bromsulphalein, and that retention of the dye also follows injections of India ink.

Three dogs were anesthetized with ether, and subjected to splenectomy. Tests were run immediately following the removal of the organ. Test readings of 20% dye in the 5 minute sample and from 5 to 10% in the 30 minute sample were obtained in all animals. This is a definite though slight retention of the dye. In sections of the liver of 2 of the dogs fixed immediately following the test, no

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[3] Saxl, P., and Donath, F., *Wien. Arch. f. inn. Med.*, 1926, **13**, 7.

[4] Shellong, F., and Eisler, B., *Z. f. d. ges. exp. Med.*, 1928, **58**, 738.

[5] Herlitz, C., *Acta Pediatrica*, 1931, **12**, Sup. 5, 1.

[6] Klein, R., and Levinson, S. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 179.

[7] Dragstedt, C. A., and Mills, M. A., *J. Lab. Clin. Med.* (in print).

demonstrable changes in the organ could be found. The third dog was tested daily following the operation. The dye clearance was again within the normal range in 48 hours.

In experiments on the reticulo-endothelial system, it has been assumed that multiple injections of blockading materials produce a progressively increasing blockade of the system until a maximum, or theoretical "complete" blockade is reached, and that these injections must be continued to maintain the blockade. Rapid recovery of function is known to follow discontinuance of the injections. Accordingly, dye tests were run following single and multiple injections of India ink and saccharated iron oxide, as recognized blockading materials. Two hours following a single injection of 40 cc. of 8% India ink, dye retentions of 25% in the first sample and 15% in the second sample were obtained. Twenty-four hours later the readings were 20% and 5% respectively. Tests run 24 hours after each of a series of daily injections of 20 cc. of 8% India ink showed a progressively increasing retention of dye with a maximum after the sixth injection of 60% and 40% in the 2 samples. Readings of 20% and 5% were found 96 hours following the last injection. The animals were not followed longer than 96 hours after stopping the injections.

Repeating these procedures with injections of 15 cc. of 20% saccharated iron oxide, less marked results were obtained. A single injection of the material produced only slight dye retention after 2 hours with normal values after 24 hours. Multiple injections of iron oxide caused only a slightly increasing dye retention although readings of 20% and 5% were obtained after 2 injections. The dye clearance was again normal after 96 hours.

In several types of acute experiments on the reticulo-endothelial system it appeared that if a rapid method of blockade could be used it would eliminate much of the labor and hazard of the multiple injection method. To this end a modified Woodyatt pump which would deliver a continuous flow of 2 cc. per minute of the blockading material, (India ink, 8%, and iron oxide, 5% or 10%), was used. Average total amounts of 150 to 250 cc. of material were injected for blockade. Bromsulphalein test readings taken at intervals of 5 minutes to 3 hours following such injections gave average readings of 60% to 80% in the 5 minute sample, and 50% in the 30 minute sample for India ink. With iron oxide, values of 40% (in one instance 60%) were obtained in the first sample and 10% to 30% in the 30 minute sample.

Summary. The observations of Klein and Levinson, that the intravenous injection of India ink in dogs causes retention of bromsulphalein are confirmed. The dye retention becomes progressively greater with repeated daily injections. These observations, in conjunction with the finding that splenectomy produces a period of temporary dye retention may be interpreted as indicating that bromsulphalein excretion is related in some way to the reticulo-endothelial system, and that the degree of dye retention may be used as a quantitative expression of the degree of "blockade" or impairment of the reticulo-endothelial system produced by blockading procedures. A marked degree of impairment, as measured by the dye retention can be produced during the course of 2 or 3 hours by a continuous injection of blockading agents. Saccharated iron oxide was found to be less effective as a blockading agent than India ink as measured by the bromsulphalein dye retention which it produces.

8570 C

Anaphylaxis in the Normal Unanesthetized Dog.

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During the course of an investigation carried out in this laboratory, anaphylaxis was induced in 61 normal unanesthetized dogs. Since references in the literature to anaphylaxis produced under these conditions in a large series of dogs are meager, it was deemed advisable to record our results.

The dogs were sensitized by injecting 5 cc. of normal horse serum subcutaneously and 5 cc. intravenously. After a 16 day incubation period the animals were given the provocative dose of horse serum consisting of 10 cc. administered intravenously.

Evaluation of the anaphylactic reaction in unanesthetized animals depends on the observation of symptoms and not on the determination of measurable responses; so it is subject to individual interpretation. This difficulty was overcome as far as possible by dividing the reactions into several classes according to their intensity, and then arbitrarily designating certain sets of symptoms as characteristic of each class. Thus all reactions are described as either slight, moderate, moderately severe, or severe. In a slight reaction the animal shows only slight transitory symptoms, little or no disturbance of respiration, never loses interest in his surroundings, and will eat meat 5 minutes after the injection. In a severe reaction the animal can not be aroused from the stuporous state in which he lies on his side and manifests marked respiratory difficulty. Moderate and moderately severe reactions are graded between these extremes. In a moderate reaction the animal, although weak and ataxic, is able to move about unsteadily under his own propulsion. In a moderately severe reaction the animal sinks to the floor but can be aroused from his stupor and made to stand on his feet.

Classification does not depend entirely on the symptoms outlined above since reactions differ qualitatively as well as quantitatively. These symptoms act merely as rough determinants, the full evaluation depending on the judgment of the observer. In a characteristic moderate reaction the dog presents the following symptoms. Following an initial progressive ataxia which is most manifest in the hind legs, the animal becomes progressively weaker until he sinks to the floor. During this period, which occupies from 2 to 5 minutes, there is an involuntary passage of urine and feces accompanied by violent retching and vomiting. After sinking to the floor the dog can walk about if placed on its feet, but soon sinks to the floor again. Respiratory symptoms usually set in about 5 minutes after the injection of the shocking dose of horse serum. They are characterized by a prolonged labored inspiration. The symptoms reach their height in about 5 minutes and then gradually abate until at the end of 30 minutes the dog is well on the way to recovery.

Of the 61 dogs 13 (21.5%) had a slight reaction, 26 (42.6%) had a moderate reaction, 10 (16.4%) had a moderately severe reaction, and 12 (19.7%) had a severe reaction. It should be noted that 63.9% of the reactions are classified as either slight or moderate. No dog entirely escaped a reaction although in 5 it was so slight as to be insignificant. Only one dog died an acute death (that is, within 30 minutes of the injection of the provocative dose of horse serum).

8571 P

Hemolytic Streptococci from Tonsils of Cow, Hog, and Sheep.

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Investigations in our laboratories have demonstrated the constant occurrence of hemolytic streptococci of the *beta* type in the human throat and tonsillar crypts. In excised tonsils of children and adults these streptococci can be isolated from the depths of the crypts in 97%,[1] often in predominant numbers and occasionally in pure culture. These organisms conform to the virulent human type in their cultural and biological reactions. In 13.8% the streptococci from these tonsils resemble the *Streptococci epidemicus,* the mucoid encapsulated streptococcus identified with the bovine mastitis responsible for epidemic septic sore throat.[2] The *Streptococcus epidemicus* recovered from milk and throat alike correspond to the human type of streptococci. Other streptococci responsible for bovine mastitis appear to be of the bovine type and apparently are harmless to human beings.

The present investigation was to determine the presence of hemolytic streptococci in the tonsils of cattle and to study their biologic properties. The tonsils were obtained from the Chicago stockyards, during the months of October, November, and December, 1935. The pharyngeal structures containing both tonsils were brought to the laboratory and cultures were made within 2 to 3 hours after slaughter.

A transverse section through the substance of the tonsil beneath the mucous membrane was made with a sterile knife and the cultures made from the depths of the exposed crypts. Usually a small amount of debris or secretion was obtained with a loop and a dilution made in sterile salt-solution. Dilution-pour-plates were examined after 24 and 48 hours at 37.5°C. The medium was infusion-agar and 5% defibrinated human blood, with ascitic fluid (20%) to render mucoid organisms prominent.[3]

The tonsil of the cow is of the tubular variety with a flat mucous surface studded with pits which lead into crypts 1 to 2 cm. deep.

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[1] Pilot, I., and Davis, D. J., *J. Infect. Dis.,* 1919, **24**, 386.

[2] Pilot, I., and Davis, D. J., *J. Infect. Dis.,* 1931, **48**, 501.

[3] Pilot, I., Hallman, B., Davis, D. J., *J. A. M. A.,* 1930, **95**, 264.

Cultures from 133 pairs revealed hemolytic streptococci of the *beta* type in 112 (84%). The hemolytic zones were clear and complete, measuring 2 to 4 cm. across. Upon further study they differed from the human streptococci. In tests for fibrinolysis, of oxalated human blood, 92 of 100 were negative. Seventy-eight of 96 strains tested hydrolysed sodium hippurate. Fermentation of trehalose and sorbitol was irregular. Forty-nine fermented trehalose, 44 did not; 52 fermented sorbitol, 46 were negative. Acid-production was consistently high, varying from pH. 4.3 to 5.0. Nine of 22 strains killed mice injected intraäbdominally in doses of 0.5 cc. of broth-culture. Of three strains examined by Dr. Lancefield, one fell in group G, one in group H, and one was not identified; none belonged in group A, the human type. These streptococci thus conform with the animal streptococci and might be designated as the bovine tonsillar type.

The hog's tonsil is considerably smaller, flat on surface with pits leading into shallow crypts. Of 106 pairs 102 (96%) were positive for *beta* hemolytic streptococci which often occurred in large numbers. In their cultural characteristics and their reactions they resembled the streptococci of the tonsils of the cow. Thirty-nine of 43 strains were not fibrinolytic for oxalated human blood. Fourteen fermented sorbitol, 26 did not; 31 fermented trehalose, 12 did not. Six of 20 strains killed mice in 24 hours in doses of 0.5 cc. Three strains sent to Lancefield fell into group C which consists chiefly of animal strains.

The tonsils of sheep were the smallest in comparison and were examined by the same technic. One hundred of 135 (74%) pairs yielded *beta* hemolytic streptococci. The colonies appeared to be smaller than those derived from the cow or hog and required 48 hours' incubation for their full growth. The hemolytic zones were wide and distinct. Study of these strains is now in progress.

In all cultures made of the tonsils of cow, sheep and hog on ascitic blood-agar, no encapsulated mucoid forms resembling *S. epidemicus* were found. The tonsils were free from gross pathological changes. The high incidence of hemolytic streptococci in such normal tonsils lends support to the conception that, as in the human tonsil, hemolytic streptococci are an essential part of the normal bacterial flora of the tonsillar crypts of a variety of animals. These apparently harmless streptococci in the throat may be responsible for disease in cattle under certain conditions. The similarity of these strains to certain hemolytic streptococci of the bovine type present in bovine mastitis[4] suggests that the oral secretions of cattle are the important source of this common infection.

Associated with the hemolytic streptococci in tonsils of cow, hog and sheep were frequently *B. coli,* staphylococci, and occasionally non-hemolytic streptococci.

Conclusions. (1) Hemolytic streptococci are frequently found in the crypts of the tonsils of cow (84%), hog (96%), and sheep (74%). They constitute part of the normal flora of the lymphoid tissue of the oropharynx of these animals. (2) The streptococci conform to the animal type and differ from the human type and the *Streptococcus epidemicus* of bovine mastitis and epidemic septic sore throat.

8572 P

Restoration of Blood Pressure and Peripheral Resistance in Sympathectomized Dogs.

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The recent treatment of hypertension by types of sympathetic surgery has created a new interest in the extent and duration of the vascular relaxation following sympathetic ganglionectomy. There has been little experimental study of the blood pressure of completely sympathectomized animals over a long period of time. Bradford Cannon[1] reported by cuff sphygmomanometer a moderate temporary fall in blood pressure immediately after complete sympathetic ganglionectomy with a final return to normal, but the time interval is not stated.

Further investigation of this problem has been made upon 15 dogs, 5 of them controls. The right thoracic chain was removed first, the left next, and the abdominal chains last at intervals of 10 days to a month. Dummy operations were done in the same order for controls. Blood pressures were measured every few days following each operation and at longer intervals later by the arterial puncture method.

The 5 control animals, 2 of which were submitted to dummy operations, have shown during intervals of from 199 to 215 days no significant change in their blood pressures. Ten dogs were put through the 3 stage sympathectomy in from 30 to 75 days. They uniformly showed no major blood pressure alteration after the first operation, a marked reduction after the second, and little additional reduction after the third. The first 4 were sacrificed after about a month of reduced blood pressure and no remnants of sympathetic chain were found. The next 6 were kept over a longer period of time and a gradual blood pressure restoration was noticed that was complete in 4 after 94 to 225 days and partial in 2 after 110 and 145 days. These dogs will be observed for a longer time.

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[4] Gibson, H. J., and Muir, R. O., *J. Hygiene,* 1935, **35**, 238.

[1] Cannon, B., *Am. J. Phys.,* 1931, **97**, 592.

In an attempt to explain this restoration, the essential factors that normally regulate blood pressure and also the possibility of the development of some hypertensive disease in these experimental animals have been considered. Circulating blood volumes of the 4 animals whose blood pressure was completely restored were measured by the Congo red plasma dye method and found approximately normal. Blood viscosities as measured by the Oswald viscosimeter were also within normal range. Since the pulse rate in these 4 sympathectomized animals averaged 14 points less than that of the control dogs, and since X-ray measurements showed no increase in heart size, it is reasonable to assume that cardiac output has not been greatly increased during the months following the last operation. Direct cardiac output determinations using the Fick formula and heart puncture were studied on 3 dogs with restored blood pressure and found to be within the normal range established in 5 control dogs. Also urine analysis and blood chemistry determinations revealed no evidence of kidney damage in the animals postoperatively. From this study it seems unlikely that there is an increase of blood volume, blood viscosity, or cardiac output to explain the restoration of blood pressure but rather that there is a restoration of the peripheral resistance. This is confirmed by the observation that these old sympathectomized animals maintain their body temperature equally as well as normal dogs when exposed to cold, while the recently sympathectomized animals had their temperatures lowered rapidly. Final consideration of the possible mechanism of restoration of peripheral resistance in these completely sympathectomized dogs will be reported at a later date.

Since the rationale of sympathetic surgery for hypertension depends more upon an elimination or lessening of vaso-pressor response to peripheral stimuli than upon a direct effect by vascular relaxation, these recovery dogs have been tested as follows: Without anæsthesia a needle connected to a recording mercury manometer was inserted into the femoral artery. Ether at 7°C. was dashed onto the opposite limb and an electric fan allowed to blow upon it. The responses in the 4 oldest completely sympathectomized animals and in 4 of the controls were compared. All 8 showed an elevation of blood pressure and heart rate together with surprise and struggle. The average increase of blood pressure over readings taken before and after the stimulus in the control dogs was 32 mm., and in the sympathectomized dogs 16 mm. Hg. The mechanism of this rise in blood pressure is not determined.

From these experiments it seems fair to assume that even a complete sympathectomy will not permanently alter the blood pressure or the peripheral resistance of normal dogs longer than 94 to 225 days.

8573 P

Characteristics of Small Colony Variants of *Shigella paradysenteriæ* Sonne and *Staphylococcus aureus.*

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A number of small-colony variants of the G-type were isolated from plates streaked with 2- to 5-month cultures of *S. paradysenteriæ* Sonne. These variants occurred irregularly and infrequently —to the extent of one per cent of the colonies counted. Cells possessing the potentiality of developing G-colonies were not encountered in 18- to 24-hour cultures. The variants produced colonies from 0.04 mm. to 0.20 mm. in diameter. In microculture the normal cell divided by a process similar to budding while the variant divided by simple transverse fission. The minimal reproductive time of the normal was about 36 minutes and that of the variants from one hour and 45 minutes to 3 hours and 54 minutes.

Reversion of the variants to the large-colony or normal form was brought about by cultivating in serum-enriched media, incubation for 5 days or by 15 to 20 serial transfers in nutrient or veal-infusion-broth. After reversion, normal biochemical, serological and cultural characteristics were reëstablished. Some strains, while believed to be true variants, could not be made to revert completely to the normal colonial size.

The biochemical activity of the variants was greatly reduced as compared to the normal. Sugars were fermented very slowly and in some cases not at all. The variants often required 10 to 30 days to produce acid in some sugar-broths. The addition of serum to the medium usually resulted in better growth and fermentation of a wider range of sugars.

Agglutination and complement-fixation tests showed most of the variants to be related to the corresponding normal strains. There existed a direct relationship between colonial size and titer with normal antiserum.

By means of their "resting cells" the comparative activities of the normal and G-colony forms of *S. paradysenteriæ* Sonne and *Staphylococcus aureus* were studied. In the presence of a hydrogen-donator, such as glucose, resting cells reduce methylene blue. The variants reduced the dye at a slower rate than did the normal cells. The time of reduction was roughly proportional to the colonial size and the concentration of organisms.

A direct relationship was found to exist betwen cataphoretic velocity and the size of the colony which the cell was capable of forming. The larger the colony the faster the velocity of the cells when corresponding normal and G-strains were compared. For instance, a normal strain of *Staphylococcus aureus,* colonial diameter 2.00 mm., had a velocity of 16.3 μ per second while its corresponding G-colony with a diameter of 0.20 mm. had a velocity of 9.5 μ per second. Those G-strains which partially reverted to the normal had a velocity between that of the normal and the variant.

The cataphoretic velocity is related to the electrical charge upon the cell and a higher velocity generally signifies a greater charge. The normal forms therefore possess a greater electrical charge than do the small variants.

Those G-strains which possessed the ability to revert to the large colonial forms were not filtrable through Berkefeld N, W or Seitz filters. One minute variant which never reverted completely passed through Berkefeld N filters 50% of the trials.

8574 C

Pressor Reactions and Bacterial Localization in Central Nervous System.

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When we produce pressor episodes in an animal body, some region of the vascular bed must be relatively anoxemic. In such regions, particularly those in which vascular anastomoses are poor, or terminal vessels exist (skin, central nervous system, special sense organs, etc.) stimulation promptly follows, the endothelium of the capillaries becomes more permeable (Landis)[1] and we may assume that bacterial adhesion can here occur to a greater extent than under normal conditions of circulation.

In general, the capillaries of the brain and the central nervous system are less permeable than those of the splanchnic bed and consequently they are less "sticky". Bacterial localization in the brain, considering the volume of the blood flow, is relatively infrequent—abscess formation certainly not common.

We were interested in the localization of bacteria in the central nervous system and for this purpose used single as well as repeated injections of pitressin (betahypophamine) to bring about periods of transient anoxemia in some of the tissues of the central nervous system. Increased stickiness and permeability of the endothelium of the blood vessels at the foci of anoxemic stimulation might enhance the localization of bacteria here as well as elsewhere.

The throats of 15 dogs were sprayed with a suspension of *B. megatherium* and *Staph. aureus* with the purpose of tracing the passage of bacteria through intact mucous membrane to the bloodstream and ultimately to the brain. The animals were divided into 3 groups of 5 each.

1. In the first (control) group the animals were destroyed 40 minutes after spraying of throat. Microörganisms were very rarely observed in the sections of the brain.

2. In the second group, pitressin was injected intravenously 10 minutes after spraying and 40 minutes later the animals were destroyed. The number of microörganisms in sections of the brain did not exceed those found in the control group.

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[1] Landis, E. M., *Am. J. Phys.*, 1927, **81**, 124.

3. In the third group, the animals had received repeated injections of pitressin at 3 and 4 day intervals. One animal received 9; one, 12; two, 17; and one, 25 injections. Ten minutes after spraying an additional intravenous injection of pitressin was given and 40 minutes later the animals were destroyed. In this third group the microörganisms were found in appreciable numbers, with the cocci forming definite groups. *B. megatherium* was not found at all in the tissues of the first 2 groups of animals, but in the third group a few single rods were found.

We were aware that the staining of bacteria in the tissues not infrequently leads to confusion and the method is open to the criticism that an object, presumably bacterial, may actually be an artifact. For this reason we gave 5 rabbits from 5 to 9 injections of pitressin intravenously at intervals of from 3 to 4 days. On the day of the last injection a suspension of *B. megatherium* and *Staphylococcus aureus,* previously stained and thoroughly washed, was injected intravenously. After one hour the animals were bled to death.

The tissues of the hippocampus major and the spinal cord were examined in frozen sections, without the use of any stain and the findings were quite similar to those of the dogs observed in the third group, namely, a considerable degree of bacterial localization in the tissues of the central nervous system.

Four normal rabbits that had received a single injection of stained bacteria, but without injection of pitressin, were treated in a similar way. No organisms were observed in the nervous tissue.

The repeated spasm of the blood-vessels of the central nervous system that follows the injection of pitressin apparently ultimately results in alterations in the tissues that can be demonstrated objectively. These changes are now being studied microscopically.

These results suggest that pressor effects may lead to the localization of bacteria in regions where vascular spasm has been particularly pronounced. The mechanism involved is probably associated with the production of focal areas of tissue (and capillary) stimulation in the regions that have been anoxemic. Here the capillaries become more permeable and adhesive, they dilate, the current is slowed and the opportunity for localization of bacteria in transit is greatly enhanced.

8575 C

Experimental Gonococcal Infection in the Chick Embryo.

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It has been found that gonococci remain viable within the interior of a hen's egg for several weeks and in certain cases as long as 3 months. Table 1 illustrates the frequency with which the organism was recovered from eggs at varying periods of time after inoculation.

TABLE I.

Time	No. eggs inoc.	No. eggs positive
1 wk.	14	13
2 ”	10	9
3 ”	7	6
4 ”	2	2
2 mo.	9	6
3 ”	6	4

Serial inoculations made from egg to egg showed that the organism also multiplied within the interior of the yolk.

Inasmuch as the embryo develops at a temperature of 37°C (and in certain instances hatches) and since the gonococcus seemed to multiply at this temperature within the egg, it appeared possible that during development the blood stream might be invaded by the organism if introduced into the yolk-sac.

Accordingly fertile eggs were incubated at 37°C and when the embryo had grown to the 12-23-day stage the yolk-sac was inoculated as follows:

A strain from a case of vaginitis in a young girl was grown on Bradford's medium and after 48 hours was washed off with one cc. of saline. A small area, one cm. in diameter, on the shell was sterilized with tincture of merthiolate and a very small opening was made through the shell with a sharp sterile probe. Using a one cc. tuberculin syringe and a 26-gauge needle, 0.1 cc. of suspension was introduced into the interior of the egg. By doing the inoculation in a dark room before an egg "candle," it was usually possible to avoid injuring the embryo. The site of inoculation was sealed with collodion and the egg returned to the incubator.

From one to 13 days later the embryos were removed, necropsied, and the yolk, heart's blood and liver were cultured. When removed, the embryos varied in weight from 7 to 18 gm. The majority had feathers. Table II shows the results of cultural studies.

TABLE II.

Egg No.	Days incubated	Days inoculated	Embryo	Cultures for GC			
				Yolk	Heart	Liver	
1	18	8	Living	+	—	—	Direct smear from eye +
2	13	7	”	+	0	—	
3	13	13	Dead	+	+	—	” ” ” ” ”
4	12	5	Living	+	0	0	
5	19	2	”	0	0	0	
6	19	2	”	0	0	0	
7	19	3	Dead	+	+	+	Direct smear from yolk and liver +
8	19	5	Living	0	0	0	
9	19	1	Dead	+	0	0	Large hem. at point of inoc. Probably fatal
10	19	2	Living	0	0	0	
11	17	4	”	+	0	0	
12	23	7	Dead	+	+	0	
13	14	12	”	+	0	0	
14	14	2	”	+	+	—	

At the time of removal of the embryos, 6 were dead and of these 4 yielded viable organisms from the heart's blood. Positive heart's blood cultures were never obtained from embryos which were alive when removed from the shell.

Anatomically, neither the gross nor the stained sections revealed evidence of tissue-reaction to the presence of the organism. Gram-stains of the sectioned material revealed occasional organisms in the liver of one embryo and in the wall of the abdominal cavity in 2 instances.

Whether invasion of the blood stream in the 4 embryos occurred before or after death could not be determined.

The use of the whole egg suggests itself for the purpose of keeping gonococcal cultures in stock and even as a method of cultivating the organism from sources such as joint-fluid. Further observations in this direction are being made.

8576 P

Rôle of Pepsin in Experimental Production of Gastric Ulcer in the Rat.*

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(Introduced by B. Kramer.)

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The rôle of gastric juice in the etiology of gastric and duodenal ulcer has been stressed. The hydrochloric acid has received the major portion of attention.[1] This investigation attempts to evaluate the relative importance of pepsin in the experimental production of gastric ulcer in the albino rat.

Buchner, Siebert and Malloy[2] reported that subcutaneous injections of histamine into fasting rats produced ulcers of the prostomach. These authors, as well as Burkle-de la Camp[3] contended that the resultant increase of both hydrochloric acid and pepsin were responsible for the observed ulcers.

It occurred to us that a more direct approach to this problem could be made with controlled feeding experiments, employing mixtures of pepsin and hydrochloric acid and hydrochloric acid alone. In such manner, we felt that the relative importance of these factors could be evaluated.

A total of 58 rats of the original Wistar strain, averaging in age 4 months, were fed the Steenbock-Bills[4] stock diet. Fresh lettuce or sliced carrots were added on feeding days. Fluids were supplied by means of stoppered flasks, with glass tube outlets, attached to the cages. The rats were then able to partake of these solutions ad lib.

The first group (1) received a 20% (by weight) mixture of granular pepsin (1:10,000 Parke, Davis and Co.*) in 0.3% solution of hydrochloric acid. The second group (2) received a 0.3% solution of hydrochloric acid alone. These solutions were freshly prepared every other day. One-half of each group (A) were fasted for 2 days and fed on the 3rd day; the control half of each group (B) had access to food at all times. The third group (3) received a 20% (by weight) mixture of inactivated pepsin (by heat) in 0.3% solution of hydrochloric acid, being fasted for 2 days and fed on the 3rd day.

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*Parke, Davis and Co. kindly supplied the pepsin used in our experiments.

[1] Dragstedt, L. R., *Surg., Gyn., and Obst.*, 1936, **62**, 118.

[2] Buchner, Siebert and Malloy, *Beitrage zur path. anat. and alleg. Path.*, 1928, **81**, 391.

[3] Burkle-de la Camp, H., *Deutsche Z. f. Chirurgie*, 1929, **220**, 31.

[4] Bills, C. E., Honeywell, E. M., Wirick, A. M., and Nussmeier, M., *J. Biol. Chem.*, 1931, **90**, 619.

In group 1A, 19 of the 20 rats, or an incidence of 95%, showed multiple ulcer-like lesions in the prostomach. In group 1B, one rat had 5 and another had only one lesion in the prostomach. The remaining 8 showed no gross changes. In group 2A only 3 animals developed gastric lesions. The other 10 showed no gross changes. In group 2B, out of 10 animals none showed any gross gastric lesions. In group 3, 2 of 10 rats (20%) developed gastric lesions.

The lesions have raised margins and umbilicated centers. They measure approximately 0.5 to 5 mm. in greatest diameter. Microscopically, the lesions simulate a localized erosive or ulcerative gastritis. These lesions are in no sense comparable to peptic ulcers in man since they occur in the proventriculus which is lined with squamous epithelium.

These experiments appear to establish that pepsin is a more important factor than HCL in the production of gastric lesions in the rat. With alternating fasting periods, 19 of 20 animals (95%) had multiple ulcer-like lesions in the pepsin group, while with 0.3% hydrochloric acid alone only 3 of 18 (16%) developed similar gross lesions. With continuous access to food, only 2 of the 10 animals had gastric lesions in the pepsin group and none in the acid group. Inactivated pepsin and 0.3% hydrochloric acid with fasting periods of 48 hours in our controls produced lesions in 20% of our animals. This almost corresponds to the incidence in group 2A (hydrochloric acid alone). The spontaneous occurrence of similar lesions in the rat is rare. In a series of 1144 rats examined by Fibiger[6] only 11 spontaneous prostomach lesions were found. Pappenheimer and Larimore[5] also reported that these rarely occurred in rats on complete diets. We found no gastric lesions in 65 control animals on various laboratory diets and fasting periods.

Conclusions. 1. Pepsin in combination with hydrochloric acid is far more effective than hydrochloric acid alone in the experimental production of ulcer-like gastric lesions in the albino rat. 2. Fasting is an important factor in the experimental production of these ulcer-like lesions.

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[5] Pappenheimer, A. M., and Larimore, L. D., *J. Exp. Med.*, 1924, **40**, 719.

[6] Fibiger, J., quoted by Burkle-de la Camp, H., *Deutsche Z. f. Chirurgie*, 1929, **220**, 31.

8577 P

The Reproductive System and Its Responses to Ovarian Hormones in Hypophysectomized Rhesus Monkeys.*

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From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

This report is based on 17 adult or subadult Rhesus monkeys which were hypophysectomized at varying stages in the sex cycle and have post-operative periods varying from 4 to 111 days. Eighteen 10-day periods of injection with oestrin (Progynon B, dihydrotheelin, Amniotin) were given and in 3 cases this was followed by progestin for 10 days (1 or 2 Rab. U./day).† Fourteen of these animals have been autopsied and the completeness of the ablation verified by a study of serial sections of the hypophyseal capsule.

The hypophysectomies were performed by the oral approach. The soft palate was incised and the hypophysis exposed by an opening through the sphenoid. The exposure of the gland with this approach is excellent, a clear view of all but the most lateral parts of the gland being secured. The penetration of the pituitary stalk through the diaphragma is clearly seen. The pars tuberalis is not removed. It is quite extensive in the monkey. The incidence of infection is low, 2 animals out of approximately 30 operated on having developed serious infection at the site of operation.

Hypophysectomy during menstruation did not prolong bleeding nor did it induce bleeding if done shortly after menstruation. Animals hypophysectomized in the middle of the cycle and showing a well developed sex-skin color showed on 2nd to the 4th day thereafter grossly evident uterine bleeding which lasted for 3 to 6 days. Bleeding was prevented by the injection of oestrin (400-500 R.U./day was given). If untreated the endometrium and the vaginal epithelium showed an involution as great or greater than after castration.

A 10-day treatment with oestrin (400-500 R.U./day) developed the involuted endometrium into the typical proliferative type

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

† We wish to express our appreciation to Drs. Schwenk of the Schering Corp. for the Progynon B and dihydrotheelin, to Dr. Morrell of E. R. Squibb & Sons for the Amniotin, and to Dr. Klein of Wilson & Co. for the material we used in making progestin.

and the vaginal epithelium became greatly thickened and cornified. In 8 cases bleeding followed cessation of treatment and in 2 cases no bleeding occurred. In those animals which bled it was always greatly delayed over non-hypophysectomized castrates, bleeding beginning on the 11th to 21st days in our series. The remaining 8 cases of oestrin treatment were either killed at the end of treatment or it was followed by progestin so that they gave no data on bleeding from oestrin withdrawal.

Following the oestrin injections 3 treatments of 10 days each with progestin have been given to 2 animals. In 2 of these treatments, given 2 Rab. U./day, bleeding occurred on the 3rd day following cessation of the injections. In the other treatment 1 Rab. U./day was given and bleeding did not follow progestin withdrawal. A typical progravid endometrium resulted from the injections.

The size of the ovaries is not greatly reduced by hypophysectomy. This we attribute to the large amount of stroma present. There is, however, a profound atresia of the follicles. As in hypophysectom-

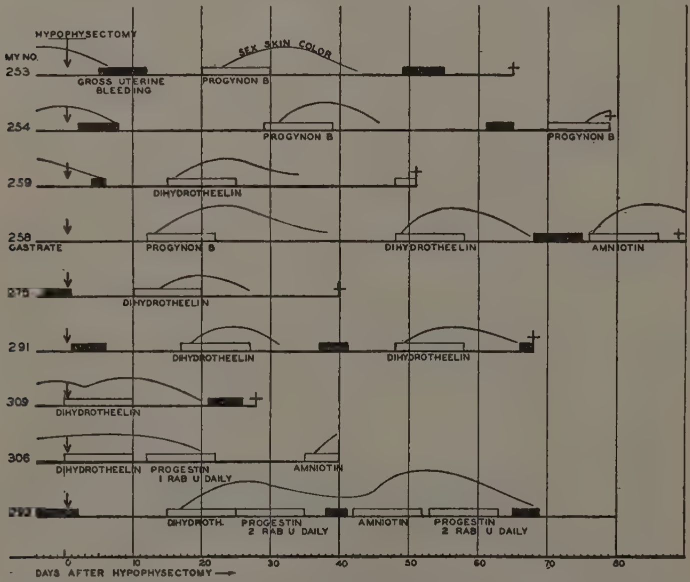

FIG. 1.

Figure showing relation of time of hypophysectomy to menstrual cycle or sex-skin color and the post-operative treatments and responses. Solid squares indicate grossly evident uterine bleeding, the open squares the time of treatment and the curved lines the color response of the sex-skin. Four hundred R.U./day of Amniotin and Progynon B was given and 500 R.U./day of dihydrotheelin.

ized rats, follicles with small antra may develop, but usually show atresia. Large follicles quickly show atresia following the pituitary ablation.

The proneness of the hypophysectomized animals to collapse from hypoglycemia makes it necessary to follow their feeding closely and injections of glucose may not infrequently be necessary as is pointed out elsewhere.

The disabilities following hypophysectomy are so widespread that it is difficult to refer the delay in bleeding after oestrin withdrawal to any specific factor. There occurs the usual atrophy of the other endocrine organs and reproductive organs, a pronounced and constant drop in blood sugar and also a pronounced drop in blood pressure and in heart rate.

8578 P

Effect of Hypophysectomy on Blood Sugar of Rhesus Monkeys.*

P. E. SMITH, LOUIS DOTTI, H. H. TYNDALE AND E. T. ENGLE.
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It has been demonstrated that hypophysectomy lowers the blood sugar in fasting dogs and rabbits. In dogs—the form in which sugar values are most extensive—the fasting blood-sugar values overlap those of normal animals and prolonged starvation is necessary to lower them to convulsive levels. Moreover, as recently demonstrated, the exposure of the hypophysis by the temporal approach, even though rapid, lowers the blood sugar nearly as much as does the removal of the gland (Chaikoff *et al.*[1]) The temporal approach has been used in the studies reported on blood sugar in hypophysectomized dogs.

The hypophysectomies here reported were done by the oral approach. The possibility of brain injury with the technique used is extremely remote due to the toughness of the diaphragma which is uninjured in the operation. In the animals reported as hypophysectomized the complete removal of the anterior and posterior lobes

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

[1] Chaikoff, I. L., Reichert, F. L., Larson, P. S., and Mathes, M. E., *Am. J. Physiol.*, 1935, **112**, 493.

of the pituitary was verified by a study of serial sections. The pars tuberalis was left intact. The material here reported is obtained from 24 normal, 9 completely and 3 partially hypophysectomized monkeys. The blood sugar of monkeys after hypophysectomy always shows such a pronounced drop that there is no overlap between the normal and operated animals after 16-18 hours starvation. Collapse due to hypoglycemia occasionally but not usually accompanied by convulsions which are never severe, happened frequently after 18 hours of starvation. This led us to reduce the fasting period to 16 hours. Even non-fasted animals may go into hypoglycemic collapse and have to be injected with glucose. Frequent and adequate feeding is necessary to maintain the hypophysectomized monkey. None of the 3 animals which were shown subsequently to be only partially hypophysectomized ever went into collapse.

The blood sugar was determined by the Somogyi modification of the Shaffer-Hartman method, reagent No. 1 being used. Each observation reported is the average value from 2 determinations on the same sample. The normal values were found to be about 10 mg. % higher in the ear than in heart blood which we attribute to the exposure to the air and accompanying concentration and also to the fact that in the drawing of this blood by this method done only in the earlier part of our work, the animals were subjected to more excitement.

The blood sugar values and their frequency distribution are shown in the accompanying chart and tables. In 3 animals which were partially hypophysectomized, the values are intermediate between those of the normal and completely hypophysectomized animals. In these cases not over one-fourth of the gland was left so far as could be determined by rough estimation from a study of sections.

In normal animals the fasting blood sugar values ranged from

TABLE I.
Blood Sugar Determinations on Heart Blood Taken 16 to 18 Hours After Fasting, Both Before and After Complete Hypophysectomy.

Monkey No.	Before Operation		After Operation	
	No. samples	Aver. mg. %	No. samples	Aver. mg. %
275	1	101	3	45
259	5	105	4	60
287	3	113	1	76
288	3	110	2	64
291	1	109	3	55
293	2	108	2	73

TABLE II.
Summary All Blood Sugar Determinations.*

	No. samples	Source blood	Aver. mg. %	ε	εm
		Normal			
Starved 18 hrs.	44	ear	120	19.6	3.0
" " "	53	heart	110	14.2	2.0
Fed	0				
		Incomplete Hypophysectomy			
Starved 18 hrs.	4	ear	93	—	—
" " "	28	heart	87	14.2	2.7
Fed	6	ear	112	—	—
		Complete Hypophysectomy			
Starved 18 hrs.	5	ear	66	—	—
" " "	33	heart	59	17.7	3.1
Fed	6	ear	94	—	—

*The formula $\epsilon = \sqrt{\frac{\Sigma d^2}{N-1}}$ was used, giving the mean deviation rather than the standard deviation (σ) because of the small number of cases studied. (Scott, *J. B. C.*, 1927, **73**, 81).

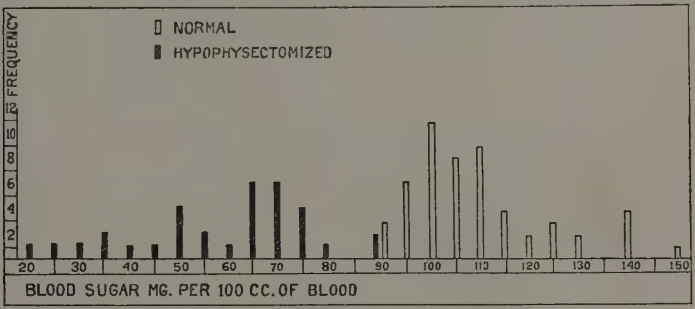

FIG. 1.
Distribution blood sugars of monkeys, starved 16-18 hours.

90 to 150 mg. %, while in the completely hypophysectomized animals the values range from 20 to 90 mg. %. The fasting blood sugar values of heart blood after hypophysectomy in the monkey average 59 mg. % (33 observations) while that of the normal animal averages 110 mg. % (53 observations).

8579 P

Response of Normal and Hypophysectomized Rhesus Monkeys to Insulin.*

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It has been shown that dogs which are hypophysectomized show an increased sensitivity to insulin (Houssay and Magenta,[1] Geiling *et al.*,[2] Chaikoff *et al.*[3]). This method was used a number of years ago in a monkey to test for the completeness of the pituitary ablation Hartman *et al.*[4]). We have tested the magnitude of the drop of blood sugar in completely and partially hypophysectomized and normal monkeys when insulin was injected after fasting. Since in half an hour after the insulin injection some of the operated animals collapsed the determinations could not be safely carried beyond that interval.

The procedure was as follows: After a 16-hour period of fasting a sample of blood was taken by cardiac puncture. Insulin (1/16 U./kilo) was then injected. Thirty minutes later a second blood sample from the heart was drawn, at which time in the collapsed hypophysectomized monkeys 50% glucose was injected into the heart to prevent death from hypoglycemia. In others 10% glucose was given subcutaneously.

The blood sugar was determined by the Somogyi modification of the Shaffer-Hartman method, reagent No. 1 being used. Each observation reported is the average value from 2 determinations on the same sample.

It will be seen from Table I that hypophysectomized monkeys are very sensitive to insulin injections. The blood sugar values of normal animals decrease by 14% 30 minutes after 1/16 unit per kilo, while the completely hypophysectomized animal shows a blood

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

[1] Houssay, B. A., and Magenta, M. A., *Comp. rend. Soc. de Biol.*, 1925, **92**, 822.

[2] Geiling, E. M. K., Campbell, D., and Ishikawa, Y., *J. Pharm. Exp. Therap.*, 1927, **31**, 247.

[3] Chaikoff, I. L., Reichert, F. L., Larson, P. S., and Mathes, M. E., *Am. J. Physiol.*, 1935, **112**, 493.

[4] Hartman, C., Firor, W. M., and Geiling, E. M. K., *Am. J. Physiol.*, 1930, **95**, 662.

TABLE I.
Effect of Injection of 1/16 Units Insulin per Kilo in Monkeys.

Condition animal	No. monkeys	No. samples	Aver. initial mg. %	Aver. mg. % 30 min. after insulin	% decrease
Normal	5	12	115	99	14
Incompleted	3	5	78	66	15
Hypophysectomized	7	11	57	37	35

sugar drop from an average initial value of 57 mg. % to 37 mg. %, a decrease of 35%.

It appears of interest that although the initial level of the blood sugar in the partially hypophysectomized animals was considerably lower than in the normals, nevertheless the percentage drop in blood sugar was nearly identical with that of the normal animals. The 3 partially hypophysectomized animals never went into collapse from the insulin injections whereas the completely hypophysectomized ones often did. In the incomplete hypophysectomies it is estimated that in no case was more than one-fourth of the normal anterior lobe present.

The blood sugar values in 6 cases of hypophysectomized animals in collapse from fasting alone averaged 29 mg. % while in 4 normal and 3 hypophysectomized animals in collapse after insulin injections the average values were approximately the same, 26 mg. %.

8580 P

Functional Auto- and Homoplastic Thyroid Grafts in the Rat.*

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Many of the attempts to graft the thyroid gland have met with little success. Manley and Marine,[1] however, reported auto-transplanted thyroids in rabbits which survived with apparently normal structure for 271 days, the animals showing "marked amelioration of symptoms of operative myxedema." Loeb[2] in a series of heterotransplants found complete early degeneration in all cases usually much before the 18th day. Marine and Rosen[3] attempted to extend the life of homotransplants in the guinea-pig by administering anterior hypophysis extract, but these thyroid grafts remained viable for only 8 days. A method for obtaining persistent functional homotransplants of the thyroid is a desirable and important aid to experimental studies of the gland and it may have clinical significance.

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council, administered by Dr. P. E. Smith.

[1] Manley, O. T., and Marine, David, PROC. SOC. EXP. BIOL. AND MED., 1915, **12**, 202.

[2] Loeb, Leo, *J. Exp. Med.*, 1920, **31**, 765.

We have been engaged in an extensive series of thyroid transplants in rats of the Long-Evans strain, which have been inbred since 1913. Autotransplants in these rats resulted in 100% viable grafts, if the thyroid was removed at 1 to 7 days of age and immediately grafted subcutaneously over the left ribs. The rats reached maturity with a normal growth curve and with no evidence of any glandular deficiency, gross or histological. The extreme ease with which viable autotransplants were obtained when infantile thyroid tissue was used prompted a subsequent series of homo-grafts.

In a series of 18 rats, homo-grafts were made as follows: (1) Reciprocal homoplastic thyroid grafts between day-old littermates. (2) Thyroids of day-old rats into thyroidectomized rats whose ages varied from 16 to 92 days. (3) Reciprocal transplants between littermates of approximately 2 weeks of age. In all but one case the grafts had taken and were functional at one month as judged by significant reactions of the host, *e. g.* rate of body growth, growth and appearance of hair, etc. Certain individuals in each group were sacrificed at this time in order that the grafted thyroids and the hypophyses might be studied cytologically.

The thyroid transplants showed an intact glandular structure, but almost invariably with a heightened epithelium and considerable colloid depletion. The cytological features, Golgi apparatus and mitochondria, were comparable to the hyperactive thyroid previously described.[4] The fact that excellent Golgi and mitochondrial preparations were obtained indicates in itself the healthy state of the thyroid tissue. There is no evidence of cytolysis or glandular absorption.

The hypophyses of the graft recipients are furthermore an excellent indication of the functional activity of the transplanted thyroids. Severinghaus, Smelser and Clark[5] have shown that marked and typical changes of the rat hypophysis regularly follow thyroidectomy and hyperthyroidism. Our homoplastic thyroid grafts maintained the hypophysis of the recipient in a normal condition. In the one thyroidectomized rat in which the graft failed to take, the typical thyroidectomy changes occurred in the anterior hypophysis. One rat, furthermore, showed hyperthyroid changes in the hypophysis, and an examination of the thyroid graft revealed an exceptionally large gland in a hyperactive state.

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[3] Marine, David, and Rosen, S. H., *Am. J. Physiol.*, 1934, **107**, 677.

[4] Severinghaus, Aura E., *Z. für Zellforsch. u. Mikr. Anat.*, 1933, **19**, 653.

[5] Severinghaus, A. E., Smelser, George K., and Clark, Helen M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1125.

Those rats which were not sacrificed at one month for cytological examination have now reached an age of from 3 to 6 months. Their appearance and reactions are completely normal. They show no indications of any thyroid disturbances. They will be under observation in experiments now in progress, at the end of which a final report on the entire thyroid transplant series will be made.

From these series of auto- and homo-thyroid grafts, it is obvious that in our Long-Evans colony of rats the thyroid of an infantile animal may be grafted into a thyroidectomized rat of any age with the expectancy of 100% viable and functional grafts, which persist indefinitely.

The next stages in the experiment will be attempts to transplant the infantile rat thyroid into rats of an unrelated strain and if these attempts are successful, heterografts will then be attempted.

8581 C

Multi-plane Chest Electrocardiography.

Joseph Weinstein. (Introduced by M. A. Rothschild.)
From the Medical Service of the Jewish Hospital, Brooklyn.

Recent investigations in the field of electrocardiography have been directed towards methods for the more accurate detection and localization of myocardial lesions. The 3 conventional leads frequently fail to detect alterations consequent to a lesion and they have recently been supplemented by chest and precordial leads introduced by Wolferth and Wood,[1, 2, 3, 4, 5] Hoffman and Delong,[6]

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[1] Wolferth, C. C., and Wood, F. C., *Am. J. M. Sc.*, 1932, **183**, 30.

[2] Wolferth, C. C., and Wood, F. C., *M. Clin. North America*, 1932, **16**, 161.

[3] Wood, F. C., and Wolferth, C. C., *J. Clin. Invest.*, 1932, **11**, 815.

[4] Wood, F. C., and Wolferth, C. C., *Arch. Int. Med.*, 1933, **51**, 771.

[5] Wood, F. C., Bellet, S., McMillan, T. M., and Wolferth, C. C., *Arch. Int. Med.*, 1933, **52**, 752.

[6] Hoffman, A. M., and Delong, E., *Arch. Int. Med.*, 1933, **51**, 947.

Wilson and his co-workers[7, 8, 9, 10] and others. However cases of acute coronary occlusion which fail to produce electrocardiographic alterations even in these leads are not an uncommon occurrence. In this communication I will discuss a method of chest lead application which I previously reported with the study of a series of normal cases.[11] My method entails the use of long linear electrodes placed parallel to and beyond the borders of the heart anteriorly and posteriorly. Various combinations of these electrodes are used in the hope of utilizing other recording planes.

The right and left arm electrode wires of the conventional leads are connected to the chest electrodes in such a manner that the current take-off is kept in the same relationship to the current direction within the heart as in lead I of the 3 standard leads. The right arm electrode wire is therefore always connected to the chest electrode which lies in closest relation to the tail of the arrow while the left arm electrode wire is connected to the chest electrode which lies in closest relation to the head of the arrow representing the heart action current direction. The size of the heart and the position of the cardiac borders are determined. The chest electrodes are placed about 2 or 3 cm. beyond the estimated position of the borders of the heart.

Flexible electrodes about ½-inch wide and 4 or more inches in length, depending on the size of the heart, are used. Coils of copper wire or suitable German silver plates are used. The electrodes should be covered with gauze soaked in warm saline solution or electrode jelly may be used. The electrodes are held firmly in place by an assistant whose hands are insulated with rubber gloves, or by a spring clamp or rubber bands.

TABLE I.

Leads	Right Arm Electrode Applied to Position	Left Arm Electrode Applied to Position
R′R	R′	R
L′L	L′	L
S′I	S′	I
R′L	R′	L
L′R	L′	R
RL	R	L

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[7] Wilson, F. N., *Am. Heart J.*, 1929-30, **5**, 599.

[8] Wilson, F. N., Macleod, A. G., and Barker, P. S., *Am. Heart J.*, 1931, **7**, 207.

[9] Wilson, F. N., MacLeod, A. G., and Barker, P. S., *Am. Heart J.*, 1932, **7**, 313.

[10] Wilson, F. N., MacLeod, A. G., Barker, P. S., Johnston, F. D., and Klostermeyer, L. L., *Heart*, 1933, **16**, 155.

[11] Weinstein, J., *Ann. Int. Med.*, 1934, **7**, 1503.

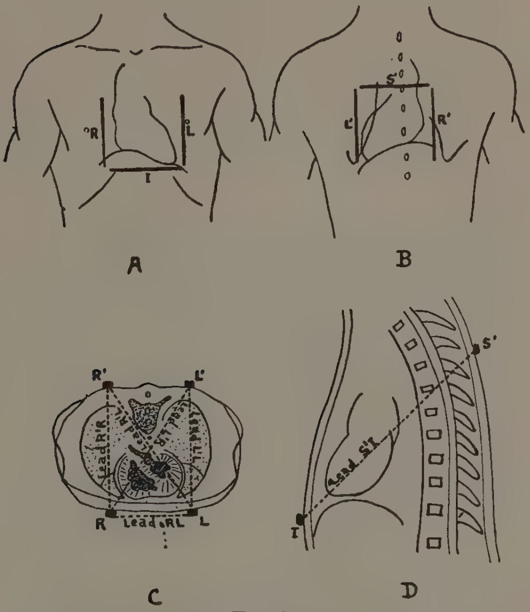

FIG. 1.

Illustration of the positions for the application of the electrodes. Their length and location are dependent on the size of the heart.

A. On the anterior chest wall: R is a vertical line beyond the right border of the heart extending from about the level of the right chondrosternal junction downward. L is a vertical line beyond the left border of the heart at the same level as R. I is a horizontal line beyond the inferior border of the heart extending from the lower end of R to the lower end of L.

B. On the posterior chest wall: Positions R′ and L′ are determined by the extension of positions R and L directly posteriorly to the posterior chest wall. S′ is a horizontal line extending from the upper end of R′ to the upper end of L′.

C and D. Cross section and lateral views of the chest illustrating the positions for the application of the electrodes and the combinations used for the multi-plane chest leads.

The electrodes are applied to the positions on the chest wall and connected in the various combinations as illustrated in Figure 1.

A study of 100 normal cases gave strikingly uniform tracings in all the multi-plane chest leads. Fig. 2 illustrates a normal case taken from this group. The standard leads, lead IV and the multi-plane chest leads are shown. The time element remains the same in all the leads. The conformation of the waves in the multi-plane chest leads resemble in most respects the waves in the standard leads. The T wave is upright, the only exceptions being in leads R′R and L′R where normally the T wave may be upright, diphasic or even inverted depending on the normal physiologic rotation of the heart as with an elevation of the diaphragm. The QRS complex may vary in direction but a prominent Q wave is not present normally in any of the multi-plane chest leads. The RT transition is under all circumstances normally on the iso-electric line.

The following cases of acute coronary occlusion, although showing comparatively normal tracings in the 3 standard leads and on lead IV, presented definite alterations in the multi-plane chest leads.

Case 97 (Fig. 3), a female 41 years of age, gave a history of an essential hypertension of many years duration. For the past year she had suffered with palpitation, dyspnoea and precordial pain on exertion requiring rest for relief. Ten days before admission to the hospital, the patient suffered a severe attack of precordial pain lasting 20 hours and requiring repeated hypodermic injections of morphine. She ran a subsequent temperature up to 102°F. She had a rapid red cell sedimentation rate and a leukocytosis of 15,800 with 84% polymorphonuclear leukocytes. Physical examination revealed a blood pressure 168 systolic and 110 diastolic, slight cyanosis of lips. The heart was enlarged to the left, the aorta percussed widened. The heart sounds were of fair quality, A_2 was accentuated, no murmurs were heard. The liver was not enlarged and there were no dependent edemas.

The standard leads and lead IV showed relatively normal tracings. The multi-plane chest leads showed an elevation of the RT segments and coronary type T waves in leads L'L, S'I and R'L, lead RL showed an elevation of the RT segment with an inversion of the T wave, leads L'L, S'I, R'L and RL showed prominent Q waves.

The patient died 7 days after this examination. Post mortem examination of the heart revealed sclerosis of the coronary arteries and an occlusion of an anomalous branch of the left coronary artery with a myomalacia of the postero-lateral wall of the left ventricle.

Case 143 (Fig. 4), a male 44 years of age, who gave a history of a hypertension of many years duration and a story of an acute coronary closure one year ago, 3 days before this examination suffered a similar attack. Physical examination revealed a slight cyanosis of the lips but no evidence of venous stasis. The heart was percussed markedly out to the left but the apex impulse was felt well within the nipple line. The radial pulses were weak. The liver was not enlarged and there was no peripheral edema. The blood pressure was 120 systolic and 84 diastolic. The temperature was 101°F. The white blood count was 18,500 with 90% polymorphonuclear leukocytes. The sedimentation rate was rapid.

The 3 standard leads and lead IV were comparatively normal with the exception of a slight slurring of the main deflections and the suggestion of a low voltage. The multi-plane chest leads revealed 3 mm. elevation of the RT segments and coronary type T waves in leads L′L, R′L and RL, leads R′R and L′R showed slight alterations in the RT transition, lead S′I remained normal.

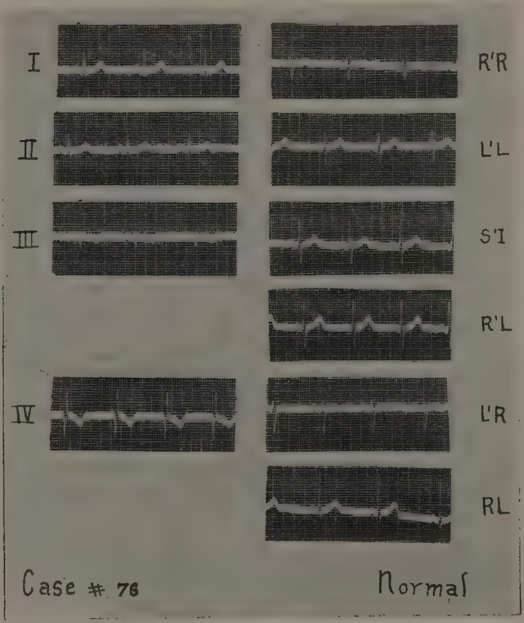

FIG. 2.

The patient died 4 days after the onset of this attack. Post mortem examination of the heart revealed a sclerosis of the left main coronary artery. There was aneurysmal dilatation involving the postero-lateral wall of the left ventricle extending down but not involving the apex. There was an area of myomalacia involving this area and the adjacent musculature extending to and involving the posterior part of the septum.

In the application of this method in experimentally produced myocardial lesions in cats,[12] a review of the records obtained in 25 experiments showed RT segment alterations in the standard leads in

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[12] Abramson, D. I., and Weinstein, J., *Am. J. Physiol.*, 1935, **3**, 1382.

only about 50% of the experiments, and lead IV showed similar changes in 66% of the series. There were instances when the standard leads showed changes while lead IV remained normal and vice versa. But two or more of the multi-plane chest leads demonstrated the lesion in all the experiments. It was further observed that when the lesion was produced on the left ventricle, anterior or posterior surface, there resulted an RT elevation in leads R'L and RL while lead L'R remained normal. When the lesion was produced on the right ventricle, anterior or posterior surface, there resulted an RT segment elevation in lead L'R and a depression in lead RL while lead R'L remained normal.

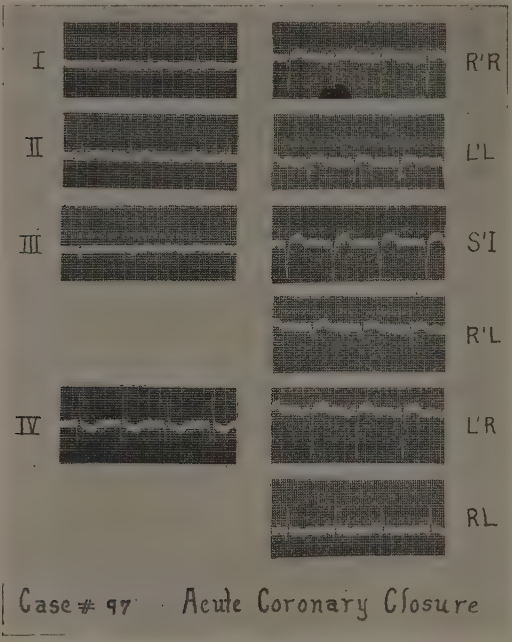

FIG. 3.

While the question of localization cannot as a rule be applied clinically because the lesion most often is not confined to one ventricle or one surface, however, the 2 clinical cases here presented conform to this classification on localization.

The multi-plane chest lead method was used in a series of 62 cases where the clinical picture suggested a coronary occlusion although the 3 standard leads and lead IV remained normal. While 12 cases of these series showed definite changes in the multi-plane chest leads, the 2 cases here discussed were the only ones where post mortem examinations were possible.

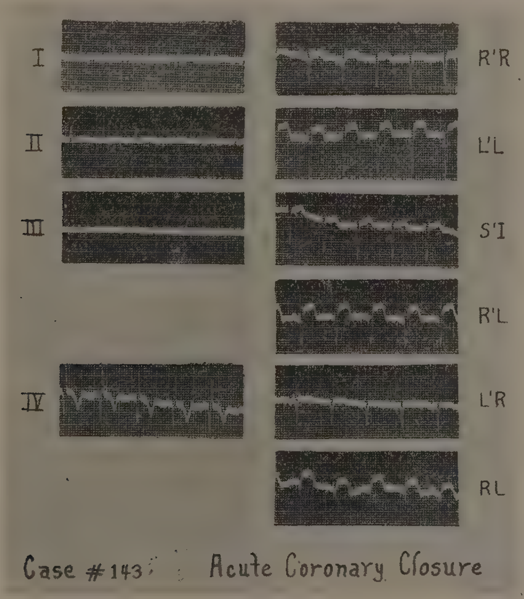

FIG. 4.

Summary. A system of multi-plane chest leads is presented. In this method long electrodes, placed parallel to and beyond the borders of the heart anteriorly and posteriorly, are connected in various combinations. Two cases of acute coronary occlusion with autopsy investigations are presented. In both cases, while the 3 standard leads and lead IV showed relatively normal tracings, the changes in the multi-plane chest leads indicated profound myocardial damage.

8582 P

Extirpation of Pituitary Gland on Arterial Blood Pressure of Dogs with Experimental Hypertension.

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The introduction by Goldblatt, Lynch, Hanzal and Summerville[1] of a reliable method by which hypertension can be produced in dogs has opened an avenue of approach of great importance to study of experimental hypertension. It consists in producing renal ischemia by means of adjustable silver clamps applied to the renal arteries.

Since the pituitary gland has been thought by some observers, notably Cushing,[2] to be involved in the genesis of hypertension in man, we have sought to find whether it also is associated in the genesis of hypertension produced by the method of Goldblatt, Lynch, Hanzal and Summerville.

Blood pressure measurements were made according to the method of Van Leersum. Arterial pressure was measured usually daily until it seemed certain that the normal variability of the pressure was known. A clamp was then applied to the right renal artery. If the rise in pressure which followed showed no tendency to disappear, hypophysectomy was performed by the subtemporal method of Sweet[3, 4] (4 animals). This method allows one to remove the whole hypophysis intact under visual guidance. Consequently it was not necessary to kill the animals to ascertain whether the operation had been complete. Failing a sufficiently steady, elevated level of arterial pressure, a clamp was applied to the left renal artery (2 animals). In one case, 5 days after the left clamp was applied, the left kidney was removed and 180 days later the right clamp tightened. In 3 animals, in which the right kidney had been clamped before hypophysectomy, clamps were applied to the left kidney after hypophysectomy. Clamps were applied to both kidneys after hypophysectomy in 4 animals. As a control the operation was performed without removal of the hypophysis in a dog in which the left renal artery was clamped. Two weeks later the gland was removed followed 38 days later by application of the clamp to the right renal artery.

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[1] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[2] Cushing, H., *Arch. Int. Med.*, 1933, **51**, 487.

[3] Sweet, J. E., and Allen, A. R., *Ann. Surg.*, 1913, **57**, 485.

[4] Sweet, J. E., *Ann. Surg.*, 1935, **102**, 1069.

Normal variations of pressure from 100 to 180 mm. of Hg were observed in our dogs. Clamping of the renal arteries produced marked elevation of arterial pressure, in some cases as high as 320 mm. of Hg.

Hypophysectomy was well borne in all of the dogs but one. The day after operation the animals ate their food and were up and about. In all but one diabetes insipidus was observed and some of the animals have become excessively fat and indolent.

The blood pressure of 4 normal dogs on which hypophysectomy had been performed appeared to be somewhat reduced below the control level, but in 2 others it was unchanged. Performance of a dummy operation without removal of the hypophysis on an animal with hypertension had no effect on average arterial pressure, but when the hypophysis was removed, arterial pressure fell from an average level of 230 mm. to 170 mm.

In the 4 dogs with induced hypertension due to clamping of the left renal artery, on the other hand, hypophysectomy caused a fall in arterial pressure; the fall began either at once or within 6 days

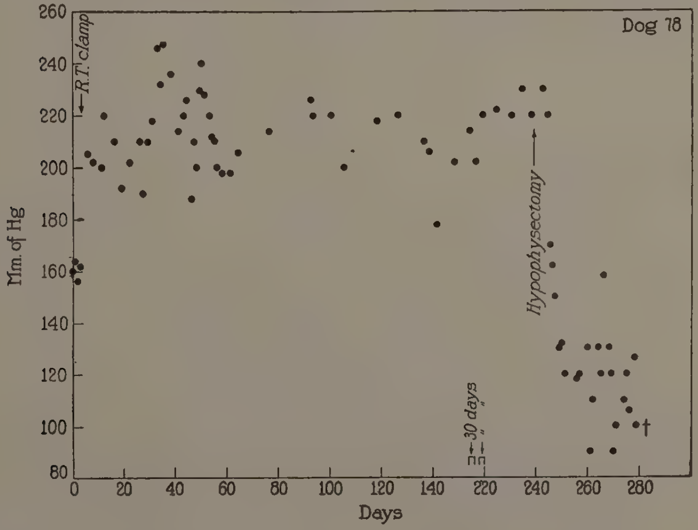

FIG. 1.

Effect of hypophysectomy on arterial hypertension in a dog (No. 78). The clamp was applied first to the right renal artery, 230 days later the hypophysis was removed.

after operation (Fig. 1). It continued until the pressure had dropped to normal. It has remained normal for 130 days in the dog which was first to undergo operation. One animal behaved differently in that the pressure fell only part of the way to normal; from a pre-operative level of 240 to 290 mm. of Hg to 160 to 200 mm. of Hg. In the 2 dogs in which both renal vessels were clamped, and after a control period of 90 days hypophysectomy performed, arterial pressure also fell to normal.

We have been less successful in keeping the hypophysectomized animals alive when the clamps were applied after hypophysectomy. Removal of the gland appears to make the animal unusually susceptible to anesthesia. Often they did not recover from the operation of clamping, a circumstance rarely occurring with normal animals or those with hypertension. Application of the clamp was usually followed by a rise in pressure (4 animals) but it may be transient. In one dog, after applying the second clamp it rose to 280 mm. The animal died 7 days after the latter operation.

Conclusions. Hypophysectomy in dogs with hypertension produced by renal ischemia (method of Goldblatt, Lynch, Hanzal and Summerville) reduces arterial pressure to about normal levels. It appears to reduce slightly the blood pressure of normal dogs. Preliminary hypophysectomy does not prevent the rise in blood pressure established by renal ischemia, but the rise tends to be transient.

8583 C

A Summary of Studies on the Effect of Ferric Chloride on Tuberculous Rabbits.*

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The object of this brief report is to describe further experiments on the effect of ferric chloride on the progress of tuberculosis in rabbits previously immunized with a strain of BCG (Bacillus Calmette-Guerin) and subsequently reinfected with a virulent bovine strain of tubercle bacilli (Ravenel). Previous studies have demonstrated that concomitantly with the accumulation of iron in tuber-

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*This work was aided by grants from the American Medical Association, Committee on Therapeutic Research, by the Committee on Grants-in-Aid, National Research Council, and by the Proctor Fund of the Harvard Medical School.

TABLE I.

Effect of Isotonic Ferric Chloride Injections on Tuberculous Rabbits.

Control Animals							Experimental Animals			
Non-vaccinated group			Vaccinated group (B.C.G.)				Vaccinated group (B.C.G.) injected with $FeCl_3$			
Rabbit No.	Wt. at beginning of exp.	Survival time after inoculation with virulent tubercle bacilli	Rabbit No.	Wt. at beginning of exp.	Amt. of saline injected	Survival time after inoculation with virulent tubercle bacilli	Rabbit No.	Wt. at beginning of exp.	Amt. of 0.25% isotonic $FeCl_3$ injected	Survival time after inoculation with virulent tubercle bacilli
	gm.	days		gm.	cc.	days		gm.	cc.	days
10-24	1550	27	9-58	2400	179	60	9-50	2530	233	97
10-96	2630	32	9-54	2770	203	75	9-48	2235	213	100
10-28	1890	35	9-47	2490		89	9-49	2525	230	100
10-97	2375	36	9-55	2505	229	93	9-56	2400	232	111
10-23	2375	43	9-45	2515		105	9-52	2425	239	122
10-13	2330	45					9-61	2025	245	270
10-22	1860	47								
10-18	3045	49								
Aver.		39				84				133

culous areas the course of the disease in rabbits is definitely retarded. This has been shown in 4 independent series of experiments.[1, 2, 3, 4, 5] The mechanism to account for this favorable effect has not as yet been elucidated. The accumulation of iron in tuberculous areas is as a rule demonstrable not by microscopic test but only in the gross when the tuberculous lung tissue is in contact with acidified $K_4Fe(CN)_6$. Experiments previously described[6] have shown that this is in large part due to considerable loss of the iron presumably during the "clearing" stage in the technique of microscopic preparation. Parallel to the deposition of iron in tuberculous areas an intracellular iron pigment is also found by microscopic examination to be deposited in the spleen, liver, and bone marrow. The pigment is indistinguishable from hemosiderin, but is not referable to hemoglobin degradation. It has therefore been termed *cytosiderin.*[6, 7, 8] The presence of this iron pigment in various organs in

TABLE II.
Summary of Studies on Effect of Ferric Chloride in Tuberculous Rabbits.

Series	Results published in	Dose of virulent tubercle bacilli	Route of inoculation of virulent bacilli	Total No. of rabbits	Aver. survival time		Increased survival time exp. animals
					Control animals	Exp. animals	
		mg.			days	days	%
1	1932	.001	intrav.	16	61	109	78
2	1933	.001	,,	20	94	135	44
3†	1936	(appx.) .01	,,	19	84*	133	58
4	1934	.05	subcut.	10	130	246	89
5‡	1934	.05	,,	10	81	198	144
					—	—	—
Aver.					90	164	82

Series 6 (1933). 16 tuberculous rabbits sacrificed between 45th and 79th day of the disease. Extent of tuberculous involvement in lungs of experimental animals considerably less than in controls.

Series 7 (1933). 19 tuberculous rabbits sacrificed between 35th and 108th day of the disease. Tubercle bacilli inoculated subcutaneously. Experimental rabbits showed definite retardation in spread of the bacilli as evidenced by extent of lesion, particularly in the lungs.

Total No. of rabbits in series: 110.

*Average survival time in non-vaccinated group of 8 control rabbits was 39 days.

†This group received a preliminary vaccination with B.C.G.

‡This group received preliminary vaccination with Cernay strain.

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[1] Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1931, **53**, 919.

[2] Menkin, V., *J. Exp. Med.*, 1932, **55**, 101.

[3] Menkin, V., *Am. J. Med. Sci.*, 1933, **185**, 40.

[4] Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 951.

[5] Menkin, V., *J. Exp. Med.*, 1934, **60**, 463.

[6] Menkin, V., *Arch. Path.*, 1935, **19**, 53.

[7] Menkin, V., and Talmadge, S. M., *Arch. Path.*, 1935, **19**, 61.

[8] Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 755.

addition to the non-pigmented form of the metal, seen in the gross within pulmonary tubercles, renders it difficult at present to account adequately for the beneficial effect of $FeCl_3$ in tuberculous rabbits. Furthermore a few preliminary experiments employing other iron compounds, or injection routes other than the intravenous one, fail to yield results identical with those obtained by the writer when large numbers of rabbits are repeatedly treated with dilute ferric chloride solution intravascularly.[9] In addition to the present set of data, a summary of all previous studies is tabulated (Table II).

A group of Flemish rabbits was immunized by BCG, by the intravenous injection of 10 mg. of the bacilli intravenously. About 2 months later these same animals were reinoculated intravenously with a large dose of a virulent bovine strain of tubercle bacilli (Ravenel). In this series the reinfecting dose of bacilli was 10 times greater (.01 mg.) than that employed in previous sets of intravenously infected rabbits. The purpose of this was to determine whether the favorable effect of ferric chloride on the experimental disease would still be appreciable with massive infection. Half of the animals were then immediately started on a course of repeated intravenous injections of ferric chloride (0.25%) for a period of 16 weeks. To avoid hemolysis, the iron chloride solution was rendered isotonic with mammalian blood by the addition of sodium chloride (680 mg. of NaCl to 100 cc. of solution). (In previous series the ferric chloride crystals had simply been dissolved in distilled water.) Half of the remaining tuberculous animals served as controls, some of which, however, received repeated intravenous injections of physiological saline. A group of control tuberculous but non-immune animals was run parallel to the immunized series. The latter succumbed at a very early stage (Table I). As in previous series and in spite of the larger intravenous inoculating dose of virulent bacilli, the immunized and iron-treated rabbits survived for distinctly longer intervals than the immunized controls (Table I). The initial weight seemed to bear no direct relationship to the survival time. Fig. 1 shows the extent of tuberculous involvement in the lungs of the longest survivors in the 3 groups of rabbits. It is clear that the lung of rabbit 9-61, the longest experimental survivor, evidently shows the least involvement, both in respect to size and amount of confluence of the lesions.

These consistent results, involving 5 independent series of experiments carried on for a period of 5 years, are now based on well

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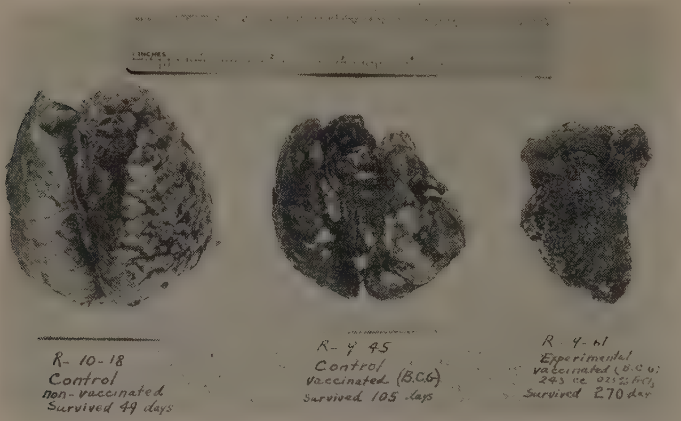
[9] Steinmann, B., *Beitr. Z. Klin. d. Tuberk.*, 1935, **86**, 84.



over 100 rabbits as summarized in Table II. The data show that dilute ferric chloride intravenously administered definitely retards the progress of tuberculosis in experimentally infected rabbits. The observations suggest possible clinical application which therefore the writer, with the permission of Dr. Donald S. King, has started at Channing Home, Boston. Several patients with far advanced tuberculosis received repeated intravenous injections of 0.0625% of isotonic ferric chloride for a few months. These injections proved completely harmless as far as noting any generalized deleterious effects. Small palpable thickenings occurred occasionally in injected arm veins. These invariably subsided within several days. The iron salt was evidently well tolerated in the concentration used.

8584 C

Fibrinolytic Activity of Beta Hemolytic Streptococci from Cow's Milk.

F. R. SMITH, C. L. HANKINSON AND C. S. MUDGE.

From the Dairy Industry Division, University of California, Davis.

One of the many unsettled problems concerning mastitis is its relationship to the organism designated as *S. mastitidis*. While the majority of workers agree that this relationship must be a very significant one, any new approach to the study of this subject should be of interest.

Tillet and Garner[1] found that many beta hemolytic streptococci from human diseases were capable of causing specific lysis of human plasma clots. Negative fibrinolytic tests were observed with other bacterial species from human sources, and strains which would actively lyse human plasma clots would not cause the dissolution of rabbit fibrin. Later Tillet[2] stated, "There is a relationship between the infectivity of beta hemolytic streptococci for man and the fibrinolytic property of the cultures."

Madison[3] confirmed the work of Tillet and Garner and[4] further tested strains from horse, rabbit, man, and hog. The lytic action of each strain was determined on fibrin from horse, hog, cow, rabbit, and man, but he did not test organisms from the cow on bovine plasma or fibrin. Thus, no conclusions could be drawn respecting the applicability of these tests to the study of *S. mastitidis*.

If the organism usually associated with infectious mastitis in the cow is able to lyse bovine fibrin, then fibrinolysis may offer a useful method of studying the rôle of this organism in mastitis.

Samples of milk were carefully taken from individual quarters: (a) udders were washed with 500 ppm. chlorine-solution; (b) first streams were discarded; (c) milk was drawn into sterile tubes, taken to the laboratory and held at 4°C. until plates could be made. Usually only 3 to 4 hours elapsed from the time of sampling to the time of plating, but in a very few cases the samples stood for 15 hours. Dilutions of 1/10 and 1/100 were plated on citrated horse-blood-agar, incubated for 48 hours at 37°C., when typical colonies were fished to blood-agar-slants.

Leukocytes were counted; unless an average of one per field (300,000 per ml.) was encountered no figures were tabulated. Chlorides were determined by the method of Hammer and Bailey,[5] and pH groupings were made according to the chart of Plastridge and Anderson,[6] using Brom Thymol Blue as the indicator.

If the leukocyte count exceeded 300,000 the milk was considered suspicious, while a count greater than 1,000,000 was tabulated as positive. A chloride content above 0.150% was looked on as either a doubtful or positive test for mastitis.

Fibrinolytic tests were made by the method of Tillet and Gar-

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[1] Tillet, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[2] Tillet, W. S., *J. Bact.*, 1935, **29**, 111.

[3] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1018.

[4] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 444.

[5] Hammer, B. W., and Bailey, D. E., Iowa Agr. Exp. Sta. Res. Bul. 41, 1917.

[6] Plastridge, W. N., and Anderson, E. O., Storrs Agr. Exp. Sta. Bul. 184, 1933.

TABLE I.

Fibrinolytic Activity of Organisms Isolated from Bovine Udders.

Tests on Milk—Source of Culture								Reactions of Pure Culture					
								Fibrinolysis of bovine plasma					
Culture No.	Leukocyte count/ml.	Beta hemolytic count	Brom thymol blue grouping	Chlorides %	History of samples	Organism isolated	pH in dextrose broth	½ hr.	3 hr.	7 hr.	8½ hr.	13 hr.	24 hr.
1, 2, 3, 5	—	300	1	.132	A-1	Strep.	4.6	—	—	—	—	—	—
4	—	300	1	.132	A-1	”	4.6	—	—	—	+	+	+
6	—	20	1	.122	B-1	”	4.6	—	—	—	—	—	—
7	—	20	1	.122	B-1	”	4.6	—	—	+	+	+	2+
8	15,000,000	10	4	.270	C-1	Staph.	Above 6.0	—	—	—	—	—	—
9	300,000	40	3	.133	C-1	”	5.8	—	—	—	—	—	—
10	300,000	40	3	.133	D-1	”	5.8	—	—	—	—	—	—
11	6,000,000	300	4	.238	C-2	Strep.	4.6	—	—	—	—	—	—
12	6,000,000	300	4	.238	C-2	”	4.6	—	+	2+	3+	3+	3+
13	—	200	1	.151	D-2	”	4.6	—	—	—	—	—	—
14	—	200	1	.151	D-2	”	4.6	—	—	+	+	2+	2+
15	—	10	1	.126	A-2	Staph.	Above 6.0	—	—	—	—	—	—
16	600,000	100	1	.100	D-3	Coccus	4.6	—	—	—	—	—	—
17, 18, 20	—	70	1	.148	B-2	Strep.	4.6	—	—	—	—	—	—
19	—	70	1	.148	B-2	”	4.6	—	—	—	—	—	+
21	8,400,000	1,000	4	.123	C-3	”	4.6	—	—	—	—	—	—
22	8,400,000	1,000	4	.123	C-3	”	4.6	—	—	—	—	—	±
23, 24	6,000,000	3,300	4	.299	C-4	”	Above 6.0	—	—	—	—	—	—
25	6,000,000	3,300	4	.299	C-4	”	4.6	—	+	2+	3+	3+	3+
26	—	30	1	.130	A-3	”	Above 6.0	—	—	—	±	±	2+
27	—	50	1	.125	B-3	Coccus	5.8	—	—	—	—	—	—
28	—	50	1	.125	B-3	Strep.	4.6	—	—	—	—	±	+
29	—	30	1	.138	E-1	Coccus	Above 6.0	—	—	—	—	—	—
30	—	10	1	.130	F-1	”	5.8	—	—	—	—	—	—
31	—	20	1	.134	B-4	Strep.	4.6	—	—	—	—	+	+

ner, using a pure culture. All of the cultures used were isolated from milk which had been subjected to the above-mentioned tests, and all cultures were tested on bovine plasma.

The table giving the results of this work is divided into two parts: (a) that referring to the milk itself, its grouping and history; (b) that referring to the characteristics of the organisms isolated. Under "history," an arbitrary grouping of the milks was made as follows:

A. Normal milk*—negative 2-months' history.
B. Normal milk—mastitis in other quarter or quarters.
C. Mastitic milk—chronic type of mastitis.
D. Suspected of mastitis.
E. Normal milk—one or more other quarters suspected.
F. Normal milk—previously suspected.

Where the letters and numbers coincide the organisms are from the same milk sample.

While no autolytic test is listed in the table, we made this test and found it was negative.

Tests were made on 42 cows. In many cases more than one sample was taken from the same quarter. Samples which contained hemolytic streptococci were isolated from normal, suspected and mastitic milk. In regard to the normal samples (A-1, A-2) only one of these (A-1) contained hemolytic streptococci in appreciable numbers. However, cows listed in the table as suspected would probably have been listed as normal if we had not tested the samples from individual quarters, because an abnormal quarter might be masked by the other normal quarters, as measured by our non-specific tests, since, as can be seen from our arbitrary definition of normal, an equivocal test could quite easily be altered to conform to these standards.

Nine of the 23 strains of streptococci showed varying degrees of fibrinolysis of bovine plasma. Of these, 2 were from normal cows, 5 from cows having mastitis in one other quarter and 2 from animals with chronic mastitis.

Some of the strains were isolated from the same sample; however, the fibrinolytic activities of these strains sometimes differed. In one case only one of 5 strains, from the same source, was capable of causing the lysis of the plasma clot.

The few organisms other than streptococci were not fibrinolytic.

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*In this article the word "normal" is arbitrarily used to designate milk having not over 300,000 leukocytes per ml., giving a No. 1 B.T.B. grouping, and having a chloride test of 0.150% or less.

The fact that all strains of streptococci from the same source were not able to lyse the clot is interesting. If this test indicates virulence, we must admit that both virulent and avirulent types of beta hemolytic streptococci may coexist in the cow's udder. This point might be proven by further studies since in this work cows considered as mastitic were probably suffering from a chronic type of mastitis. In acute cases, one would expect a much higher percentage of fibrinolytic strains if there is a correlation between fibrinolysis and virulence.

The occurrence of lytic strains in cows which we class as normal does not deny the possibility that the test is of value as an indicator of virulence. Ayers and Mudge[7] isolated *S. mastitidis* from cows they believed to be normal. Plastridge and Anderson[8] do not believe these cows are normal, but that they merely temporarily fail to give the usual non-specific tests. These cows, however, may often harbor streptococci, which, possibly, may be virulent.

There is considerable evidence that *S. mastitidis* causes bovine mastitis; still there is the possibility that it is merely an opportunist. By analogy with properties of human streptococci, we do think there is some possibility that fibrinolysis of bovine plasma, by beta hemolytic streptococci from bovine udders, may be a measure of pathogenicity.

Conclusions: 1. Some beta hemolytic streptococci from milk are capable of causing the lysis of clotted bovine plasma. 2. Organisms from cows that are negative to non-specific tests for mastitis may produce this fibrinolysis.

8585 P

Growth Inhibitor in Liver Tissue.

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Liver has been known for a long time to contain substances which inhibit growth and migration of cells from explants. Walton[1] observed that extracts of liver had an inhibiting effect on tissue cultures, in contrast to all other tissue extracts tried. Lynch[2] and Lewis[3] noticed that no cells migrate from cultures of chick embryo liver after the sixteenth day, and it has been suggested that the inhibitor in liver may be responsible for this effect. Despite the great amount of work that has been done on extracts and substances which increase growth of tissues *in vitro* or make possible their prolonged cultivation, relatively little attention has been paid to inhibitors. The following is a report of investigations made in this laboratory on the inhibiting power of liver extract.

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[7] Ayers, S. H., and Mudge, C. S., *J. Infect. Dis.*, 1922, **31**, 40.

[8] Plastridge, W. N. and Anderson, E. O., Storrs Agr. Exp. Sta. Bul. 195, 1934.

[1] Walton, A. J., *J. Exp. Med.*, 1914, **20**, 554.

We have used, in general, first explants of embryonic and sarcomatous tissues, grown by the usual coverslip or Carrel flask techniques in solid plasma media. In order to get a quantitative measure of inhibition, we have compared the average radial growth in a test solution at a given time with that in a control medium consisting of plasma and Tyrode's solution, using tissues explanted at the same time from the same embryonic organ. Of course, Parker[4] has shown that the growth rates of fibroblasts from different parts of the same organ may vary; we have found, however, that by using series of 5 or more explants a growth index can be obtained which does not vary more than 10 or 15% from series to series, or from day to day in the same series.

When fresh, sterile liver tissue is passed through a fine press and the pulp is shaken with double its volume of Tyrode's solution and centrifuged, a reddish, turbid solution is obtained which, when diluted with equal parts of plasma, inhibits growth of fibroblasts to the extent of 75 to 85% of the growth seen in Tyrode's solution alone: in other words, the average growth radius is 15 to 25% of the average radius of growth in the control. In dilutions of 1:6 or 1:8, inhibition of fibroblasts amounts to 30 to 50% and in higher dilutions there is no significant effect. This inhibition has been demonstrated using saline extracts of rat, mouse, and chicken livers, and is not present in extracts of chick embryo liver before the fifteenth day. Extracts of rapidly growing hyperplastic rat liver inhibit in the same degree as those of normal rat livers. The saline liver extracts with which we have dealt have a chloride content of about 0.5%, a nitrogen content of 4 to 5%, of which nine-tenths is protein nitrogen, and a potassium content of less than 0.2%. There is no inhibition of cultures grown in Tyrode's solution modified to contain this amount of potassium as KC1. The pH of the extracts is from 7.3 to 7.5.

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[2] Lynch, R. S., *A. J. Anat.*, 1921, **29**, 281.

[3] Lewis, W. H., in E. V. Cowdry's *General Cytology*, Univ. of Chicago Press, Chicago, 1924, p. 401.

[4] Parker, R. C., *J. Exp. Med.*, 1933, **58**, 401.

Shaking the saline extract with ether for a sufficiently long time to precipitate most of the turbid material appears to destroy the inhibitor in large part, although there is slight inhibition in the ether-soluble fraction, corresponding in all probability to the mild inhibition which Carrel[5] has found in the lipoid fraction of serum. Precipitation of the turbid material by acidifying to a pH of 5.0 to 6.0 leaves a supernatant fluid which lacks any marked inhibitory action; in some instances, when the precipitate can be made to go back into solution, it is as inhibitory as the original extract. Oxygenation of the solution and evaporation of it to dryness, do not destroy its inhibitory power, and the inhibitor is stable at 50°C. for 30 minutes and is partly destroyed at 75°C. for the same length of time. Heating to 100° destroys it completely.

Ultrafiltration of the saline extract through a collodion sac yields a non-diffusible residue which is highly inhibitory, while the filtrate, tested on subdivided cultures which have acquired a constant growth rate, stimulates considerably better growth than is produced with Tyrode's solution alone (due, probably, to the presence in it of nutrient substances).

Addition of alcohol to the saline extracts up to 75% by volume, which precipitates most of the proteins, leaves a supernatant fluid which contains the inhibitor, although some of its potency is lost in the process. Seventy per cent alcohol extracts of liver pulp, when evaporated and taken up in Tyrode's solution, are inhibitory, although not in quite as high dilution as are saline extracts of liver. Such alcoholic extracts have been made from chicken, rat, lamb, calf, and steer livers, and when made isotonic and adjusted to a pH of 7.4, have always been found to be inhibitory. When obtained in this form, the inhibitor is found to diffuse through a collodion sac. The inhibitor, in both saline and alcoholic extracts, is removed by Lloyd's reagent and is not removed by shaking with large quantities of blood charcoal. In alcoholic extracts, it is partly precipitated by 40 to 60% acetone and partly soluble in 60 to 80% acetone. The inhibitory action of acetone precipitates is lost, however, when they are purified by reprecipitation from 55% alcohol.

The inhibitor is most effective on fibroblasts, and has been found to have an equal effect on various sorts of embryonic fibroblasts and on the malignant fibroblasts of mouse sarcoma 180. It is effective on homologous and heterologous tissues alike. It appears to have some inhibiting effect on the migration of monocytes, and has little effect on epithelium. The life-span of inhibited cultures does not seem to be altered and rare mitoses may be seen, but the radius of growth is greatly diminished and the cells (especially in cultures of sarcoma) are smaller.

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[5] Baker, Lillian E., and Carrel, Alexis, *J. Exp. Med.*, 1925, **42**, 143.

Further investigations now in progress are planned to determine the exact nature of the growth-inhibiting property of liver tissue.

8586 C

Growth of Human Nervous System. III. Relations between Cerebral Surface, Volume and Length.

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From the Graduate Faculty and the Institute of Child Welfare, University of Minnesota.

The human cerebrum, excluding its ventricular system, is a solid; and it is possible by the study of its measurements to determine to what degree, if any, the growth of this structure follows the usual geometric laws of the interrelation of dimensions of solids with simple and regular form.

If these dimensions approach the geometric rules of dimensionality, it is to be expected that the following relations may be approximated:

$$CV\ (=)\ k_1CL^a,\quad CS\ (=)\ k_2CL^b,\quad CS\ (=)\ k_3CV^c,$$

and also the converse expressions. In the above general formulae CV is cerebral volume, CL cerebral length, and CS cerebral surface. The k's are constants to be empirically determined, and the other lower case letters represent powers also to be empirically determined. For objects of simple and regular geometric form it is to be expected that *a* will approach 3; *b*, 2; and *c*, 2/3.

We have tested these assumptions by fitting curves for several of these dimensions as determined from a series of 20 cerebri, ranging from about 5 cc. to over 1,000 cc. in volume and from slightly less than 4 fetal or lunar months to about 50 years in age.[1] All curves were fitted by the method of average logarithms.[2]

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[1] For the data see Hesdorffer, M. B., and Scammon, R. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 415.

[2] See Lipka, J., Graphical and Mechanical Computation, New York, 1918, p. 128 *et seq.*

Figure 2 shows the relation of the volume of the cerebrum to its maximal length. The empirical formula obtained is:

$$CV = 0.29(CL)^{2.91}, \tag{1}$$

where CV is cerebral volume in cc. and CL is cerebral length in cm. The algebraic sum of the differences between the observed and calculated values is 1,110.1 cc., the mean deviation without regard to sign is 55.6 cc., and the mean relative deviation is 15.6%.

Figure 3 shows the relation of "free" surface of the cerebrum

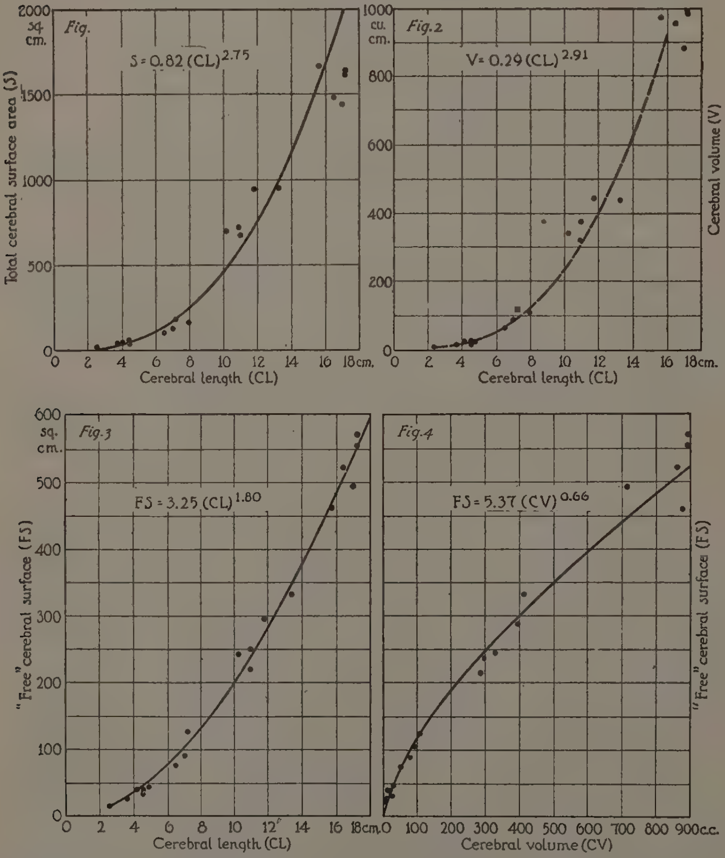

FIG. 1 to 4 (inclusive).

Graphs of the interrelations of human cerebral surface, volume and longitudinal diameter.

to cerebral length. The empirical expression for this relationship is:

$$FS = 3.25(CL)^{1.80}, \tag{2}$$

where FS is "free" cerebral surface in sq. cm. and CL is cerebral length in cm. Here the sum of the residuals is 276.3 sq. cm. The mean deviation of observed from calculated values is 13.8 sq. cm., and the mean relative residual is 10.4%.

The relation of "free" cerebral surface to cerebral volume (Fig. 4), empirically determined, is:

$$FS = 5.37(CV)^{0.66}. \tag{3}$$

The sum of the differences between the observed values and those obtained by this expression is 329.6 sq. cm. The mean absolute deviation is 16.5 sq. cm., and the mean relative deviation is 7.1%.

The relation of "total" cerebral surface (CS) to cerebral length is shown in Fig. 1 and is expressed by the empirical formula:

$$CS = 0.82(CL)^{2.75}. \tag{4}$$

The sum of the differences between the observed values and those obtained by this expression is 2,746.3 sq. cm. The mean absolute deviation is 137.3 sq. cm., and the mean relative deviation is 21.5%.

Since the value of the "total" cerebral surface may be approximated by a constant of cerebral length raised to nearly the third power and cerebral volume may also be approximated with a somewhat similar power, it follows that volume and "total" area may approach a simple rectilinear relationship. Testing this supposition by fitting the same observations on "total" cerebral area to cerebral volume, by the logarithmic method, we obtain:

$$CS = 3.26(CV)^{0.90}. \tag{5}$$

Resorting to the method of correlation, we find that the coefficient of correlation (r) is 0.99 ± 0.003, and the formula for the line of regression is:

$$CS = 0.59 + 1.64(CV). \tag{6}$$

The results of these several procedures seem to bear out in general the speculations stated at the beginning of this paper. The values for the exponents *a*, *b* and *c*, instead of being 3, 2 and 2/3, respectively, are, when empirically determined, 2.9+, 1.8+ and 0.66+. These are not far from the assumed values when the variable nature of the material, the technical errors in obtaining the data, and the method of calculating the empirical expressions are considered.

The notable exception to these approximations of the rules of dimensionality is the "total" surface of the cerebrum. This value increases throughout the period under consideration not as the two-thirds power of the cerebral volume but almost directly proportional to the volume; and not as the square of cerebral length but almost as its cube.

Some of the relationships of cerebral dimensions thus determined are illustrated graphically by the histogram forming Fig. 5. This consists of 3 series of spheres arranged vertically in order of the following ages: fetus of a (calculated) age of 3.7 lunar months, newborn (mean of 2), and adult (mean of 4 ranging from 26 to about 50 years of age). In each instance the cerebral value represented is considered as unity at 3.7 lunar months and the succeeding members of the series are computed as multiples of this first one.

The first (left) column (a) represents the changes in observed volume of the cerebral hemispheres between early fetal life and maturity, the spheres representing only the reduction of observed volumes to spheres of equal volumes.

The second (middle) column (b) shows 3 spheres calculated

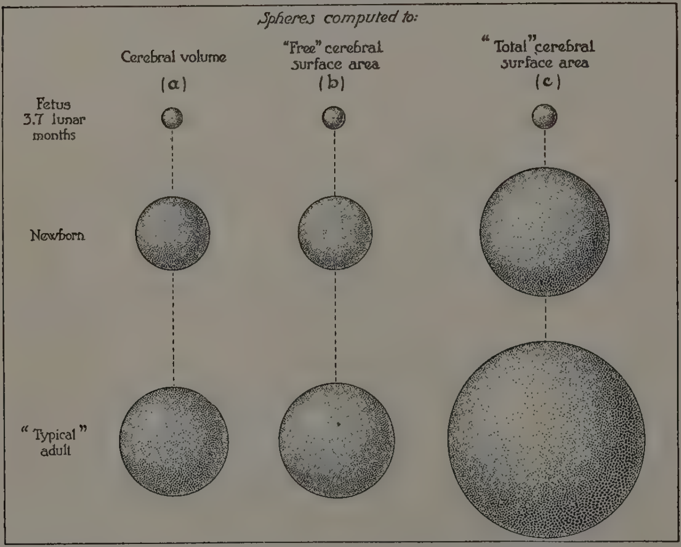

FIG. 5.
Drawing of a series of spheres illustrating in diagrammatic form the changes in relationship of the volume, the "free" and the "total" surface of the human cerebrum in prenatal and postnatal life.

from the observed "free" surface area of the cerebral hemispheres, and the third (right) column (c) represents spheres calculated from observed "total" cerebral surface.

This pictorial representation brings out the main points indicated by geometric and analytic methods. The "total" cerebral surface departs from the simple course of geometric increase approximated by the other 2 measures some time before birth, and this divergence is even more marked by the end of the developmental period; a divergence that seems obviously associated with the formation of the cerebral fissures.

8587 P

Glucose Absorption and Glycogen Formation in the Hypophysectomized Rat.*

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The following studies of glycogen formation and glucose absorption have been carried out in the hope of partially elucidating the phenomena of abnormally low glycogen levels in the fasting hypophysectomized rat.

In these experiments the control rats were between 45 and 54 days of age at the time of sacrifice. The hypophysectomized† rats were of a similar age group at operation and were sacrificed 25 to 30 days after operation; those upon which glycogen determinations were done were sacrificed 21 to 23 days after operation. Glucose absorption was determined by the method of Cori[1] after feeding approximately 2½ cc. of a 35% solution of glucose. Previous to the experiment all rats were fasted for 24 hours in cages provided with wire screen bottoms. Tissues for glycogen determination were removed under amytal anaesthesia and glycogen determined by the cold KOH method of Cori.[2] Liver glycogen figures are for fermentable reducing substances; muscle glycogen figures are the average of duplicate determinations of both gastrocnemius muscles and represent total reducing substances. All glucose determinations were done by the Shaffer-Somogyi[3] method.

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* Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins and Mrs. Gordon Kahn, of Los Angeles.

† Hypophysectomies were done by the parapharyngeal route and completeness of the operation was checked at autopsy in each case.

[1] Cori, C. F., *J. Biol. Chem.*, 1925, **66**, 691.

[2] Cori, G. T., *J. Biol. Chem.*, 1932, **96**, 259.

In confirmation of the observations of Phillips and Robb[4] glucose absorption rates in the hypophysectomized rats were found to be about 35% below normal (Table I). These animals absorbed 141 and 135 mg. per hour per 100 gm. body weight as compared with 223 and 211 mg. absorbed by the controls. There was no overlapping of the 2 groups and probable error values show this difference to be statistically significant.

TABLE I.
Mg. of Glucose Absorbed per 100 gm. Body Weight per Hour.

	1 hr. absorption	2 hr. absorption
Controls	223 mg. ± 7.7 (13)*	211 mg. ± 6.4 (14)
Hypophysectomized	141 mg. ± 4.1 (15)	135 mg. ± 4.4 (14)

*No. of animals in the group.

TABLE II.
Glycogen Levels in Fasted and Glucose-Fed Normal and Hypophysectomized Rats.*

		Glucose absorbed in 2 hr. per 100 gm. body weight	Blood sugar	Liver glycogen	Muscle glycogen
Normal Controls	(11)†	Unfed	80 mg. % ± 1.5	23 mg. % ± .12	502 mg. % ± 4.5
Hypophysectomized Controls	(10)	”	51 ” ” ± 1.3	15 ” ” ± .69	322 ” ” ± 7.8
Normal Glucose-Fed	(6)	412 mg.	140 mg. %	1348 mg. %	673 ” ”
Hypophysectomized Glucose-Fed	(6)	247 ”	111 ” ”	194 ” ”	378 ” ”

*All figures in this table are mean for group with probable errors indicated where the group is sufficiently large.
†No. of animals in group.

Table II shows the glycogen and blood sugar levels in 24 hours fasted normal and hypophysectomized rats as well as in a second series of fed rats. These data show not only that the glycogen levels of fasting hypophysectomized rats are abnormally low (this is especially true of the muscle glycogen) but that there is a smaller than normal increase following glucose feeding. The hypophysectomized rats deposited 5.9 mg. of new liver glycogen per 100 gm. of body weight (2.4% of the absorbed glucose) as compared with 60 mg. of new liver glycogen per 100 gm. of body weight (14.5% of the absorbed glucose) deposited by the controls. If it be assumed that muscular tissue represents 50% of the live weight of the rat and that glycogen is uniformly distributed, it can be calculated from the above data that the normal rats deposited as muscle glycogen 20.8% of the absorbed glucose while the hypophysectomized rats deposited as muscle glycogen only 11.3% of the absorbed glucose. While the assumptions involved in this method of calculation of muscle glycogen increases may be open to question in the hypophysectomized rat, it appears probable that hypophysectomized rats form proportionately less glycogen from the absorbed glucose than do normal animals.

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[3] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

[4] Phillips, R. A., and Robb, P., *Am. J. Physiol.*, 1934, **109**, 82.

8588 P

Carbohydrate Levels in Fasted and Fed Hypophysectomized Rats.*

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In an inquiry as to the nature of the hypoglycemia and low glycogen stores found in completely hypophysectomized rats, the following experiments have been undertaken: (1) the comparison under standard conditions of the carbohydrate levels of normal, hypophysectomized† and partially hypophysectomized rats when fully fed and when fasted 8 and 18 hours; and (2) the preparation of curves showing the changes in carbohydrate levels in these animals following the feeding of uniform carbohydrate meals.

All rats used were young males 50-70 days of age, the experimental animals 20-30 days post operative. Sodium amytal was used as anesthetic for obtaining samples. Muscle glycogen figures for each animal were the averages of 2 separate determinations, and the liver glycogen values included only fermentable reducing substances. The carbohydrate meals were given in the form of cornstarch, in 50% suspension in water, fed by stomach tube without anesthesia.

It was found that when the hypophysectomized animals were

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* Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins and Mrs. Gordon Kahn, of Los Angeles.

† The parapharyngeal approach was used and the completeness of the operation was checked at autopsy in all cases.

fully fed (taken directly from their cages) all carbohydrate levels were within normal limits, but that when they were fasted, these levels fell rapidly, as shown in Table I. Each figure in this table represents the decrease in carbohydrate content as per cent of the average levels found in the fully fed animals.‡

TABLE I.
Decrease in Carbohydrate Levels During Fasting of Normal (N) and Hypophysectomized (H) Rats.

	8 hr. Fast		18 hr. Fast	
	N	H	N	H
Liver Glycogen	27	95	96	99
Muscle ”	8	24	6	41
Blood Glucose	20	49	32	54

Values for similar series of operated controls, in which one-third or more of the anterior pituitary remained *in situ* and including several cases with various degrees of brain injury, did not differ from the normal; they are not included in this table.

That chronic inanition did not produce similar results was shown by the fact that normal rats kept on the same amounts of daily rations as were consumed by the hypophysectomized rats had fasting carbohydrate levels within normal limits.

When 1 gm. of starch was fed to rats fasted 18 hours, it was found 4 hours later that none of the levels in the completely hypophysectomized rats were as high as those in the normal animals so treated. When, however, an additional gram of starch was fed 4 hours following the first, thus providing enough carbohydrate and allowing sufficient time to overcome the initial fasting deficits, muscle glycogen and blood glucose values were easily brought to normal; but liver glycogen values even in this case increased much more slowly. This failure to form liver glycogen in the usual amounts from ingested carbohydrate may be explained in large part by low absorption rates, the rates of digestion and absorption of starch, as well as of glucose having been determined to be about 30% below normal in hypophysectomized rats. Calculation§ of the approximate increase in body carbohydrates due to feeding equal amounts of starch shows that the figures obtained in these series do not by themselves indicate the participation of other factors than those mentioned above; but their operation may not be ruled out. If, as Fisher and Pencharz[1] have found, a larger proportion of carbohydrate is oxidized by hypophysectomized rats following glucose feeding, this process also would be expected to decrease the amounts of carbohydrate stored in the liver under these conditions.

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‡ Six to twelve animals were used in every group. The average coefficient of variation (standard deviation in % of the mean) in all groups was for blood glucose 12%, for muscle glycogen 13% and for liver glycogen 50%; the differences between the average values for normal and hypophysectomized animals were significant in all fasted series.

§ Based on averages for groups of 8-13 animals in each case.

Although normal high carbohydrate levels in blood and muscle and moderately high liver glycogen values (maximum average 49% of normal) were obtained by feeding standard carbohydrate meals, these levels differed from the normal in that they were not maintained for any length of time, falling even while absorption was still continuing. The levels were followed for 36 hours after the initial feedings of one gm. of starch to normal and 1 and 2 gm. to hypophysectomized rats, determinations being made at 4, 8, 12, 24 and 36 hours, and 6-10 animals being used for each point on the several curves; the results amply confirm the conclusion previously drawn from work on fasted animals that hypophysectomized rats lose body carbohydrates at a much greater rate than do normal animals.

8589 C

Cataract- and Dermatitis-Producing Nutritional Factors.

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Cataract has been produced experimentally by parathyroidectomy, pancreatectomy, vitamin G-low diets,[1] lactose-rich[2] and galactose-containing[3] diets. In human subjects dinitrophenol has been reported as likewise cataract-producing. The difficulty of reconciling these diverse precipitating conditions is great, and in fact may not be possible. However, it is interesting to speculate upon the possible interrelation of the 2 obviously nutritional factors, vitamin G (B_2) and lactose.

The delay in, or interference with, complete absorption accompanying the presence of large amounts of lactose or galactose in the intestine and the frequent gastro-intestinal absorption difficulties in clinical conditions associated with vitamin G deficiency, for example pellagra, pernicious anemia and sprue, offer a possible common avenue of attack upon the problem. The present study was planned to test the bearing of such absorption-disturbances upon the production of cataract, of dermatitis and of growth-abnormality.

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[1] Fisher and Pencharz, PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 106.

[1] Day, P. L., and Langston, W. C., *J. Nut.*, 1934, **7**, 97.

[2] Mitchell, H. S., and Dodge, W. M., *J. Nut.*, 1935, **9**, 37.

[3] Mitchell, H. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 971; Yudkin, A. M., and Arnold, C. H., *ibid.*, 1935, **32**, 836.

The diets used are of the usual vitamin-testing type containing casein purified by repeated extraction with 60% alcohol and varying chiefly in the carbohydrate fraction and in the supplement. The formula of the diets follows:

	Cornstarch	Lactose
Casein	18	15
Salt Mixture	4	4
Butter Fat	8	
Crisco		9
Codliver Oil	2	2
Raw Cornstarch	68	
Lactose		70

As source of vitamin B a concentrated rice-polish extract (tiki-tiki) is fed in the amount of 0.06 gm. daily to each rat. This extract is not wholly lacking in the G factors but contains relatively small amounts of them. When fed to 5 rats during a preliminary test on cornstarch-containing diet at a level 10 times (0.6 gm.) that employed in this experiment, definite and in some cases marked dermatitis was developed although growth was promoted at the rate of 8 gm. per week, slightly better than that seen in a parallel series of animals receiving daily 0.075 gm. of a potent brewery-yeast. The latter animals, however, showed practically no dermatitis.

That the amount of vitamin B afforded by 0.06 gm. daily of this extract was sufficient for the animals in this experiment was shown by the 4-week failure in growth-improvement of 4 rats given additional and excessive doses of crystalline B_1 when they began to fail on the cornstarch-containing diet from lack of the G factors. The average weekly gain of this small group was 2 to 3 gm., the same as that of the larger group receiving no additional B.

Further to decrease the possible vitamin G-content of the carbohydrates used, the cornstarch and lactose were spread out in thin layers and exposed to bright diffused daylight and for part of the time to direct sunlight for 8 to 10 days. This was done because of the well established light lability of the flavins.[4]

Certain animals from each litter were given 0.5 gm. untreated

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[4] György, P., *Biochem. J.*, 1935, **29**, 767.

Northwestern brewery-yeast instead of the tiki-tiki in addition to the basal diet. These groups served as presumably normal controls receiving adequate amounts of the B and G factors.

Comparison of 4 groups was thus possible, (1) those receiving the basal diet containing raw cornstarch and the yeast-supplement, (2) those receiving the same diet but with tiki-tiki instead of the yeast, (3) those receiving the basal diet containing lactose and the yeast-supplement, and (4) those receiving the lactose-diet with tiki-tiki instead of yeast. All animals were taken at weaning and the litters divided among the experimental groups. They were examined regularly twice a week for the appearance of cloudiness, rings or striæ in the eye lens and for scaliness, scabs, loss of hair, or other evidences of abnormality. Three different lots of animals were used, but the results were so similar that they are reported together in Table I. For convenience these groups are designated as lactose-G-low, lactose-G-rich, cornstarch-G-low and cornstarch-G-rich.

TABLE I.
Relation of Lactose and Vitamin G (B_2) to Cataract- and Dermatitis-Production in Rats.

Diet	Lactose G-low	Lactose G-rich	Cornstarch G-low	Cornstarch G-rich
No. of rats	10*	14	12	12
Duration of exp., wks.	12-15	12-15	12-15	12-15
Aver. weekly gain, gm.	8	10	3	17
Incidence of cataract, %	100	85	0	0
Time of appearance of cataract, days	21-35	60-84	—	—
Dermatitis	none	none	severe	none

*3 rats died after 5 weeks.

The lactose-fed controls grew at about 60% the rate of the cornstarch-fed controls, a condition found previously by Koehler and Allen[5] in a comparison of regrowth of starved rats on lactose as compared with sucrose and glucose, and by Mitchell[6] on young rats. Occasional attacks of diarrhea were seen in all the lactose-fed rats, particularly during the first weeks of feeding. Usually these attacks lasted only a day or two. The feces were never of the refective type. Coprophagy has not been noted in any of the animals, all of which have been kept on wide mesh screens. The work of Guerrant, Dutcher, and Tomey[7] on the effect of lactose in diets deficient in the whole vitamin B complex (including G) indicated only a slight resistance to the progress of the deficiency when the feces of the rats on such a diet were fed to them. The overwhelming influence of the lack of vitamin B in that case undoubtedly obscured whatever effect might have been produced upon the G-deficiencies.

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[5] Koehler, A. E., and Allen, S. E., *J. Nut.*, 1934, **8**, 377.

[6] Mitchell, H. S., *Am. J. Physiol.*, 1927, **79**, 542.

[7] Guerrant, N. B., Dutcher, R. A., and Tomey, L. F., *J. Biol. Chem.*, 1935, **110**, 233.

The vitamin G low-cornstarch rats grew very little and developed severe cutaneous lesions, alopecia, scabs, and incrustations about the eyes and ears. On the other hand, the vitamin-G-low lactose rats grew nearly as well as their controls and showed normal skin- and fur-condition at all times. There was an exception in the case of 3 rats of one group which grew poorly, had more or less continual diarrhea, and died after 5 weeks, although no dermatitis occurred.

Mature bilateral cataracts occurred in all of the lactose-G-low animals including those which died at 5 weeks. The time of occurrence of the cataracts varied from 21 to 35 days. In the yeast- and lactose-fed group, 85% of the rats developed cataract but after a longer time, 60 to 84 days. None of the cornstarch-fed animals have shown cataract.

Obviously the same deficiency cannot account for the dermatitis and the cataracts here reported. Whatever nutritional failure is caused by the lactose involves no dermatitis and indeed prevents it.

There is a possibility that the lactose prevents the absorption or allows the destruction in the intestine of the cataract-preventing factor. To test this, 4 rats fed the lactose-G-low diet were given a pure lacto-flavin preparation supplied by Dr. S. Lepkovsky. Two of these animals were fed 20 gamma of this preparation daily by mouth and 2 given the same amount by subcutaneous injection. All 4 of these animals developed cataract at about the same rate. The rate of increased body-growth has been different, however. The 2 rats fed with flavin have gained 85 gm. in 12 weeks while the 2 injected have gained 112 gm. in the same time. This would seem to indicate some intestinal loss. Since the rats were on the deficient diet for 2 weeks and may have had incipient cataract before the flavin-administration began, it cannot be said definitely that the flavin failed to prevent cataract.

Four rats fed the G-low cornstarch-diet were also fed or injected for 6 weeks with flavin with complete cure of all symptoms of dermatitis and with no differences in growth, the average gain being 12 gm. per week in both cases. Evidently no absorptive loss of flavin occurs on these diets. The dermatitis produced by this particular vitamin G-deficient diet is completely cured by lacto-flavin, as contrasted with the negative results of flavin-administration for the cure of so-called rat-pellagra by György,[8] Chick, Copping, and Edgar,[9] and others.

8590 C

X-Ray Diffraction Patterns from Reprecipitated Connective Tissue.

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For a number of years[1] it has been known that tendon gives excellent crystalline reflections. Recently[2] large spacings have been observed which provide strong evidence for the view that collagenous structures may be fibrous arrays of true crystals composed of very large molecules.

Most forms of tendon from adult animals cannot be put into solution without obvious chemical disintegration. The work of Nageotte and of others[3] has, however, demonstrated that optically active fibrous precipitates can be obtained from weakly acid solutions made from the tail tendon of adult rats and from many connective tissues of foetal animals. The optical appearance of these precipitates suggests that they are at least similar to the tissue from which they were dissolved. We have carried out a series of experiments to determine: (a) whether reprecipitated tendon is really crystalline and (b) whether its X-ray diffraction pattern is like that of the original tissue.

Reprecipitated connective tissue was obtained according to the procedures outlined by Nageotte. Pieces of freshly excised tail tendon from adult rats were placed in dilute (1 to 25,000) acetic acid. In such acid the tendon swells and a portion of it dissolves to form a clear but highly viscous solution. The reprecipitated tissue is obtained from this solution by salting out with 5% NaCl. Series of X-ray diffraction studies have been made of a number of these preparations. Some of these diffraction photographs were made with the usual short crystal-to-film distances; others were made with very small slit systems and with the large camera radii needed to record spacings of the order of 100 A. Diffraction patterns have been photographed and measured from (1) the original tail tendon after drying over P_2O_5, (2) reprecipitated tissue dried under tension, (3) the extracted tendon dried under tension. These photographs and typical measurements made upon them (Table I) prove that reprecipitated tissue shows exactly the same X-ray diffraction lines as the initial tendon. Furthermore if the precipitate is carefully dried under stretch it shows the same type of preferred orientation as the original tendon. Drawings of the most conspicuous features of patterns of initial and reprecipitated tendon are reproduced in Fig. 1. Except that the orientation is poorer in the reprecipitated sample, as shown by greater length of the reflection arcs, the diagrams are the same. Photographs from carefully dried bits of extracted tendon are essentially the same as those of the original dried tissue.

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[8] György, P., *Biochem. J.*, 1935, **29**, 741.

[9] Chick, H. J., Copping, A. M., and Edgar, C. E., *Biochem. J.*, 1935, **29**, 722.

[1] See for example, Katz, J., Die Röntgenspektrographie als Untersuchungsmethode, Berlin, 1934, p. 189.

[2] Wyckoff, R. W. G., Corey, R. B., and Biscoe, J., *Science*, 1935, **82**, 175; Clark, G. L., Parker, E. A., Schaad, J. A., and Warren, W. J., *J. Am. Chem. Soc.*, 1935, **57**, 1509

[3] Nageotte, J., and Guyon, L., *Compt. rend. Assoc. Anatomistes, Bruxelles*, 1934, 25-28 Mars.

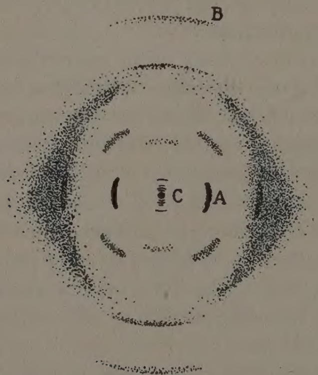

FIG. 1a.

A drawing showing essential features of the X-ray diagram of dried rat tail tendon.

These experiments demonstrate that reprecipitated connective tissue prepared according to the procedures of Nageotte is crystalline with the same X-ray diffraction pattern as the original tendon. Such a reprecipitated tissue thus can differ only slightly if at all from the original either in chemical composition or in crystalline array. This conclusion is important because it means that suitably made collagen solutions may be expected to contain substantially the same large molecules that have gone to make up the original connective tissue. A study of such solutions together with a further examination of X-ray crystal diagrams can hardly fail to throw light upon the nature of these molecules.

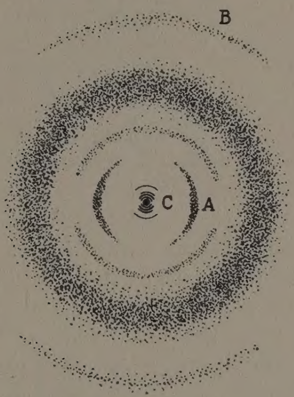

FIG. 1b.

A similar drawing of reprecipitated tendon dried under stretch. The groups of lines lettered A, B, and C in both drawings and in Table I can be more accurately measured than most other reflections in the original photographs.

TABLE I.

Reflection*	Intensity	(1) Original tendon	(2) Reprecipitated tissue	(3) Extracted tendon
B	medium	2.88 A	2.88 A	2.84 A
A	strong	10.66	10.75	10.71
C	very faint	33.3	32.7	†
	faint	54.6	53.1	55.3
	strong	70.1	71.4	71.4
	medium	109	103	109

*See Fig. 1.
†Too faint for measurement.